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Study of glycyrrhizic acid contents from *Glycyrrhiza glabra* populations in Iran and their relation with environmental factors

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Abstract. *Rezaei S, Nejadsattari T, Assadi M, Khavarinejad RA, Mehregan I. 2017. Study of glycyrrhizic acid contents from Glycyrrhiza glabra populations in Iran and their relation with environmental factors. Biodiversitas 18: 212-220.* Glycyrrhizin (glycyrrhizic acid) is an important chemical composition of licorice (*Glycyrrhiza glabra* L.) from which is 50 times sweeter than sucrose. In this study, the amount of glycyrrhizic acid was determined in populations collected from 11 different locations in Iran (NW to NE) by HPLC method from two varieties of this species; *Glycyrrhiza glabra* varieties *glabra* and *glandulifera*. Our results showed the highest content of glycyrrhizic acid was from Naghadeh (NAQ, NW Iran) population for *G. glabra* var. *glandulifera* and Azadshahr population (AZD, NW Iran) for *G. glabra* var. *glabra*. Lowest amounts of glycyrrhizic acid were detected in Jajrood (JAD, N Iran) population for *G. glabra* var. *glandulifera* and Hashtrud (HSD, N Iran) population for *G. glabra* var. *glabra*. Significant correlations were observed between the amount of glycyrrhizic acid and some morphometric parameters, soil and environmental factors. The amount of glycyrrhizic acid showed positive correlation with phosphorous ($P \leq 0.05$ and $R^2 = 0.155$) and negative correlation with pH ($P \leq 0.01$ and $R^2 = 0.348$) and calcium carbonate ($P \leq 0.01$ and $R^2 = 0.507$). Higher temperature resulted in increasing the amount of glycyrrhizic acid. Based on morphometrical data, the plants with thinner rhizomes would have more glycyrrhizic acid. Our results also showed that glycyrrhizic acid content was not related to taxonomic classification of this species.

Keywords: Ecological factors, glycyrrhizic acid, HPLC, licorice

INTRODUCTION

The genus *Glycyrrhiza* L. (Fabaceae; English: "liquorice" or "licorice") consists of about 30 perennial species worldwide (Minglei et al. 2008). Liquorice has long been used as a medicinal herb against a wide range of diseases from the common cold to liver problems in both Eastern and Western medicine (Olukoga and Donaldson 2000; Kokate et al. 2003). In 18th century Nepali philosopher Joseph Danzly introduced Licorice. Carl Linnaeus first introduced *Glycyrrhiza* with three species *G. glabra* L., *G. echinata* L. and *G. hirsuta* L. (Fiore et al. 2005). It traditionally belongs to the tribe Astragalae of the subfamily Faboideae (Rechinger 1984). Glycyrrhizic acid, a triterpenoid saponin, is the most important phytochemical compound obtained from rhizomes and roots of *Glycyrrhiza glabra* L. (IHIDMA 2002). It has also been obtained from other species such as *G. uralensis* Fisch., *G. inflata* Batalin., *G. aspera* Pall., *G. korshinskyi* Grig. and *G. eurycarpa* P. C. Li., which are generally referred as licorice (Nomura and Fukai 1998; Ammosov and Litvinenko 2007). *Glycyrrhiza glabra* and *G. uralensis* are allowed to use as licorice and licorice powder (Komiyama 2001). Rechinger introduced 6 species of *Glycyrrhiza* in his "Flora Iranica" (Rechinger, 1984). *Glycyrrhiza glabra* is usually represented by its two main varieties in Iran: 1) *G. glabra* var. *glabra,* known as "Persian or Turkish licorice" and 2) *G. glabra* var. *glandulifera* (Waldst. & Kit.) Boiss., known as "Russian licorice" (Fenwick et al. 1990; Nassiriasl and Hosseinzadeh 2008).

The genus *Glycyrrhiza* includes rhizomatous perennial herbs with yellow or blue to mauve flowers. Leaves are imparipinnate, lanceolate and alternate with 4-7 paired plus a single terminal leaflets. The calyx is companulate, bilabiate and bearing glandular hairs. The bracts are small and caduceus. The fruit is a smooth, glandular or echinate legume. As the most-known species of the genus, *G. glabra* is a perennial herb, 30-60 cm height. The leaves are lanceolate in outline, with 5-9 pair elliptic leaflets. Inflorescence is 5-9 cm long raceme. Each flower has 5 calyx teeth ca. 3 mm long and blue to violet corolla, 9-11 mm long. The fruit is red-brown legume, 15-25 mm long and 4-5 mm in diameter, glandular (sometimes bristly) or eglandular (Davis 1965; Rechinger 1984).

Different pharmacological activity had been recorded for *Glycyrrhiza.* It has been used against chronic hepatitis (Iino et al. 2001), as anti-inflammatory (Ohnishi et al. 2011; Kim et al. 2012; Ni et al. 2013), anti-bacterial (Ates and Turgay 2003), antioxidant (Sheela et al. 2006; Amirghofran 2010; Fu et al. 2013), antitussive (Anderson and Smith 1961), antiulcer (Tsai and Chen 1991; Krausse et al. 2004), antifungal (Alonso 2004), antitumor (Sheela et al. 2006; Amirghofran 2010) and hepatoprotective (Dhiman and Chawla 2005; Kim et al. 2009). Glycyrrhizic acid is the major constituent of licorice root capsule (LRC) as a premium herbal dietary supplement, and is widely used

against digestion problem, as relieving stomach cramps and immune booster in China (Yang et al. 2012). Different analytical methods have been reported for measurement of glycyrrhizic acid in plant extracts including high performance liquid chromatography (HPLC) (Yang et al. 2011; Seo et al. 2012), high performance thin layer chromatography (HPTLC) (Gantait et al. 2010), gas chromatography mass spectrometry (GC-MS) (Guillaume 1999; Lisec et al. 2006), thin-layer chromatographic (TLC) (Yang et al. 1991; Faisal et al. 2009), and capillary electrophoresis (Chen and Sheu 1993). Amount of glycyrrhizic acid in the plant is highly variable (Xie et al. 2010; Alam et al. 2014; Zhou et al. 2015).

The aim of this study was to evaluate the amount of glycyrrhizic acid in different populations from two varieties of *G. glabra* (var. *glabra* and var. *glandulifera*) from northwestern and northeastern Iran and to determine any possible relationship between these amounts and different environmental or edaphic factors.

MATERIALS AND METHODS

Plant preparation

Rhizomes (and roots) of *Glycyrrhiza glabra* were dug up from 11 different localities in northwestern, north and northeastern Iran where they grow wild in different ecological conditions (Figure 1 and Table 1). They were collected between June and November 2014. All of the samples were cleaned, cut into smaller pieces, then dried at the 25 degree centigrade (room temperature) and powdered using electric mill.

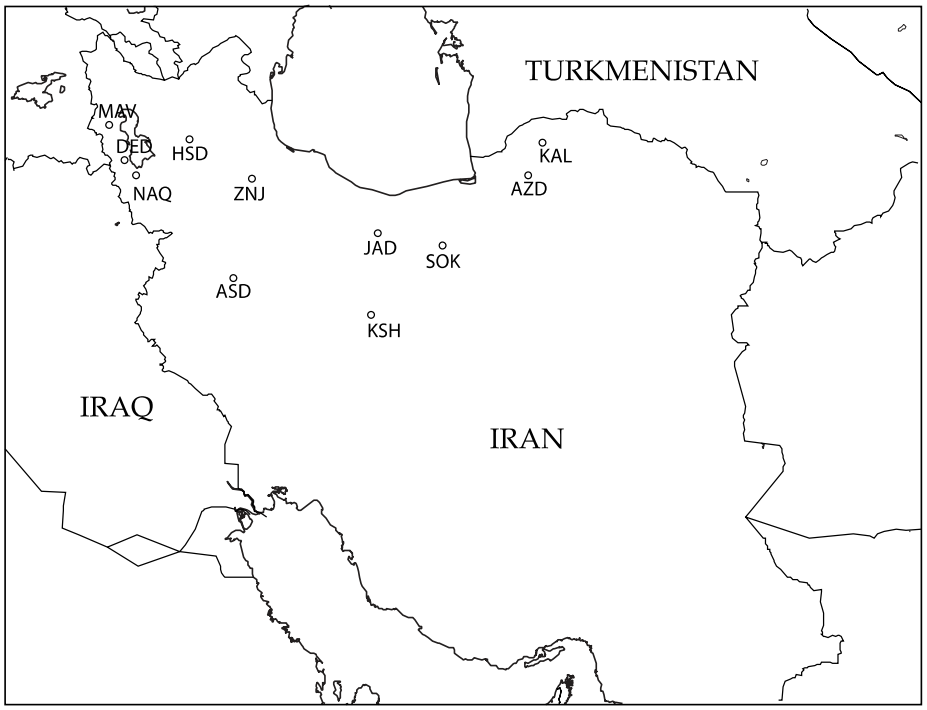

Figure 1. Localities of collected plant materials examined in this study (abbreviations and more details in Table1)

Table 1. List and localities of collected plant material examined in this study. For each population the scientific name of *G. glabra* variety (*glabra* or *glandulifera*) is given

Pop. no.	Pop. code	Collection site	Locality	var.	Lo. (°E)	La.(°N)	Alt. (m)
P1	AZD	Azadshahr	Iran: Golestan, 7 km to Azadshahr	*glabra*	55.14	37.8	87
P2	KAL	Kalaleh	Iran: Golestan, crossroad Mashhad-Kalale	*glabra*	55.28	37.18	170
P3	KSH	Kashan	Iran: Isfahan, Tehran to Isfahan road, 5 km to Kashan	*glandulifera*	51.20	34.1	986
P4	HSD	Hashtrud	Iran: East Azerbayjan, Hashtrud	*glabra*	47.2	37.24	1527
P5	JAD	Jajrood	Iran: Tehran, Jajrood	*glandulifera*	51.57	35.47	1672
P6	DED	Dizaj-e Dowl	Iran: West Azerbayjan, 50 km to Naqadeh, Dizaj-e Dowl	*glandulifera*	45.19	37.14	1320
P7	ZNJ	Zanjan	Iran: Zanjan, 15km to Zarin abad	*glandulifera*	48.155	36.330	1577
P8	SOK	Sorkheh	Iran: Semnan, 15km to Semnan, Sorkheh	*glandulifera*	53.05	35.25	1243
P9	NAQ	Naqadeh	Iran: West Azerbayjan, Naqadeh	*glandulifera*	45.33	36.58	1287
P10	MAV	Mavana	Iran: West Azarbayjan, Mavana	*glandulifera*	44.470	37.340	1591
P11	ASD	Asadabad	Iran: Hamadan, 15km to Hamadan, Asadabad	*glandulifera*	48.300	34.90	1870

Note: Pop.: population, var.: variety, Lo.: longitude, La.: latitude, Alt.: altitude

Sample preparation

In this step, 1g licorice powder being extracted with 50 ml ethanol/water (30:70, v/v) dissolved and then resulted powder was put in the water bath at 50°C for 60 min. 40 ml from this extract was transferred into 50 ml falcons and then filtered by a 0.2 µm syringe filters (Minglei et al. 2008).

HPLC analysis

High Performance Liquid Chromatography was performed on a Smartline® HPLC series (KNAUER, Germany) consisted of a Smartline® S-1000 pump, S-5000 manager with degasser and a S-2500 programmable UV detector with column C18 (150 × 4.6 mm). The mobile phase was methanol/water (70:30, v/v, containing 1% acetic acid). Flow rate was adjusted at 1.0 ml/min. After sample filtration with 0.45 µm diameter filter, 20 µl of each sample was injected (three times for each sample).

Standard curve preparation

Glycyrrhyzic acid ammonium salt (Sigma-Aldrich) was used as the standard. After filtrations with 0.45 µm diameter filters, four different concentrations of Glycyrrhyzic acid ammonium salt (50, 100, 200 and 300 ppm) were prepared and injected to HPLC apparatus.

Soil analysis

Soil samples from each population were collected at 20-30 cm depth near the roots. Soil characters were determined using following different methods. The soil texture was determined using Bouyoucos Hydrometer Method (Gee and Bauder 1979). The acidity rate (pH) and the electrical conductivity (EC) were measured using a portable CPD-65N multi-meter (ISTEK, South Korea). Atomic absorption spectroscopy (AAS) was used for measuring the Ca^{2+}, K^{+} and Na^{+} (Pybus et al. 1970). A modified Walkley and Black method was used to determine the amount of organic carbon (OC) content (Allison et al. 1965). Chlorine in the soil was measured with Ion chromatography (IC) method (Khym 1974). Amount of $CaCo_3$ as the total carbonates included in 100g of dry soil was determined using Calcimeter Bernard method. Olsen method was used for determination of Phosphor content (Nelson and Sommers 1982). Amount of Nitrogen was determined using Kjehdal method by converting the various nitrogen forms into NH_4^{+} (Bremner and Mulvaney 1982).

Ecological data

The samples were taken from different localities (Table 4) and their climate data including precipitation, average, maximum and minimum temperatures were taken from the website www.en.climate-data.org.

Morphometrical analysis

Seven morphological characters including length and width of the leaves and legumes, the number of seeds in each legume, seed and rhizome diameter (Table 5) were measured for at least five individuals from each population. The measurements were made using a digital Vernier caliper.

Statistical analysis

Resulted data were analyzed by SPSS v. 22 (IBM Inc, Chicago, IL). Kolmogorov-Smirnov test was performed to test the normality of frequency distributions. One-way ANOVA was used to compare the means of normal distributions. Duncan test was used to determine the differences in morphometrical data. Pearson's correlation analysis was performed to determine the degree of correlation between different factors. Populations were classified using Hierarchical cluster analysis (HCA) with Average-linkage method and standard Euclidean coefficient. Principal component analysis (PCA) was carried out based on the relative contents of ecological and morphometry data from different populations as dependent variables (Farag et al. 2012).

RESULTS AND DISCUSSION

HPLC analysis

Figure 2 shows the calibration curve made by plotting the different concentration of the standard glycyrrhizic acid mono ammonium salt. The HPLC chromatogram of the standard glycyrrhizic acid mono ammonium salt shows the maximum absorption at about 6th minutes after injection (Figure 3).

As shown in Table 2 and Figure 4, the amount of glycyrrhizic acid were variable in different localities; the highest amount of glycyrrhizic acid in *G. glabra* var. *glabra* was observed in AZD population (231.63 mg/l) and the lowest amount was observed in HSD population (130.95 mg/l). For *G. glabra* var. *gladulifera*, the highest amount was recorded in NAQ population (1328.4 mg/l) and the lowest one was achieved in JAD (53.75 mg/l). Amount of Glycyrrrhizic acid contents in ZNJ (var. *gladulifera*) and AZD (var. *glabra*) were nearly equal. Terefore, we could not separate two varieties based on their amount of glycyrrhizic acid.

Environmental data and soil analysis

Results of soil analyses showed that the highest EC amount belonged to SOK populations (5.8 ms/cm, Table 3) and the lowest one belonged to ASD population (1.159 ms/cm). HSD population demonstrated the highest pH amount (pH = 8.22) and MAV population showed the lowest amount (pH = 7.4). Most of localities had soil with clay-sand texture. SOK was interesting to show the highest amounts of Ca^{2+} (10.3 meq/lit), Na^{+} (25.9 meq/lit), Cl^{-} (22.6 meq/lit) and the lowest amounts of OC (0.21%), N (0.029%) and P (3.12 ppm). HSD showed the highest amount of CaCo3 (17.8%) and the lowest amount of silt (15.3 %) (Table 3).

The highest amount of average temperature was observed in KAL and AZD in N of Iran (> 28 °C) and the lowest one observed in KSH (13.1°C). MAV had the maximum annual precipitation (39.6 mm) and the lowest one observed in KSH (10.6 mm) (Table 4).

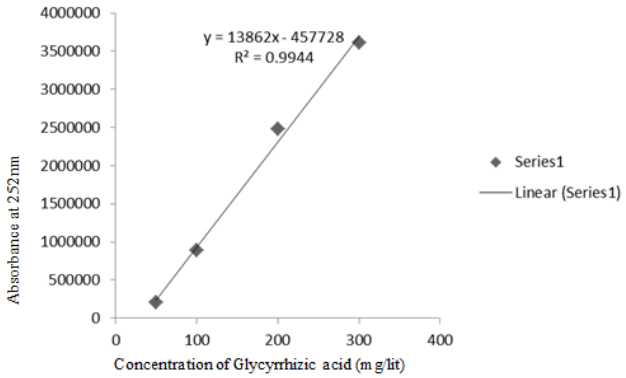

Figure 2. The calibration curve made by plotting the different concentration of the standard glycyrrhizic acid mono ammonium salt.

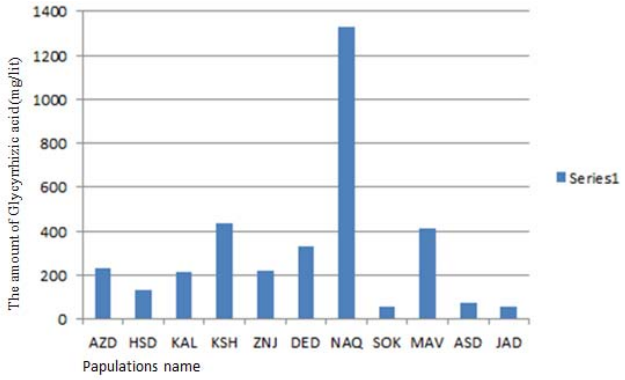

Figure 4. The amount of glycyrrhizic acid in different papulations.

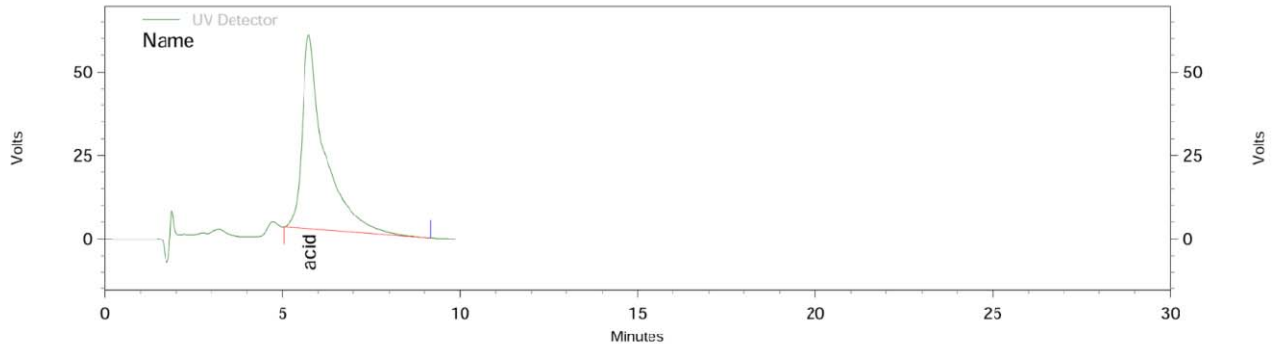

Figure 3. Standard chromatogram of glycyrrhizic acid.

Table 2. The amount of glycyrrhizic acid in different localities

Variety	AZD Glb.	HSD Glb.	KAL Glb.	KSH Glf.	ZNJ Glf.	DED Glf.	NAQ Glf.	SOK Glf.	MAV Glf.	ASD Glf.	JAD Glf.
Glycyrrhizic acid	231.63 ±21.4^{d}	130.95 ±7.5^{e}	211.37 ±77.7^{d}	437.9 ±42.3^{b}	221.41 ±9.95^{d}	328.5 ±13.2^{c}	1328.4 ±77.7^{a}	56.03 ±0.81^{f}	412.2 ±1.86^{b}	71.68 ±2.06^{e,f}	53.75 ±3.64^{f}

Note: Glb.: *Glycyrrhiza glabra* var. *glabra.* Glf.: var. *glandolifera*. AZD: Azadshahr, KAL: Kalaleh, KSH: Kashan, HSD: Hashtrud, JAD: Jajrood, DED: Dizaj-e Dowl, ZNJ: Zanjan, SOK: Sorkheh, NAQ: Naqadeh, MAV: Mavana, ASD: Asadabad. ^{a,b,c,d,e,f}: Means in the same column with different superscripts differ ($P \leq 0.05$)

Table 3. Results of analyses of soils collected from different localities

Pop. code	Ec (ms/cm)	pH	Ca^{2+} (meq/lit)	Na^{+} (meq/lit)	Cl^{-} (meq/lit)	$CaCo_3$ %	Sand %	Silt %	Clay %	Texture	OC %	N %	P (ppm)	K (ppm)
AZD	1.64	8.05	3.4	8.6	7.4	15.9	37.8	35.3	26.9	Clay silt	0.48	0.053	5.17	251.8
KAL	3.27	8.02	6.9	12.7	6.4	15.5	33.8	51.3	14.9	Clay silt	0.52	0.063	7.82	235.3
KSH	1.951	7.9	3.5	8.3	7.8	14.8	53.8	27.3	18.9	Claysand	0.34	0.038	3.78	141.2
HSD	2.93	8.22	4.1	15.7	13.4	17.8	77.8	15.3	6.9	Sandclay	0.26	0.034	3.55	178.2
JAD	1.188	7.91	1.5	5.1	6	16.9	61.8	19.3	18.9	Claysand	0.45	0.051	4.82	241.9
DED	1.765	7.81	3.1	7.9	6.2	13.4	65.8	27.3	6.9	Claysand	0.42	0.051	7.07	184.3
ZAJ	1.873	7.9	4	8.5	7	16.3	73.8	19.3	6.9	Claysand	0.31	0.039	5.11	223.2
SOK	5.8	8.06	10.3	25.9	22.6	16.1	65.8	27.3	6.9	Claysand	0.21	0.029	3.12	189.2
NAQ	3.09	7.6	7.8	13.5	10.8	12.8	73.8	21.3	4.9	Sandclay	0.38	0.045	6.51	211.9
MAV	1.52	7.4	3.2	7.8	5.8	13.5	77.8	15.3	6.9	Sandclay	0.51	0.059	7.02	255
ASD	1.159	7.91	2.2	6.1	7.6	15.2	53.8	31.3	14.9	Claysand	0.39	0.041	4.19	202.5

Note:Pop.: Papulation, AZD: Azadshahr, KAL: Kalaleh, KSH: Kashan, HSD: Hashtrud, JAD: Jajrood, DED: Dizaj-e Dowl, ZNJ: Zanjan, SOK: Sorkheh, NAQ: Naqadeh, MAV: Mavana, ASD: Asadabad

Morphometric features

Morphometric data including the means and standard deviations for length and width of the leaves, length and width of the legumes, number of seeds per legume and seed diameter are shown in Table 5. Duncan test showed that the highest and the lowest amounts of rhizome diameter were observed in SOK (5.03±0.03) and DED (3.16±0.01), respectively. ZAJ population had the longer leaves (37.14±2.15) and the shorter ones were recorded in JAD (28.36±1.98). The higher amounts of leaf width were observed in MAV population (15.56±1.12) and the lower amounts were observed in JAD (4.53±1.20). The longer legumes were observed in SOK (31.29±1.13) and the broader legumes were recorded in DED (6.81±0.62). MAV population had the shorter legumes (14.70±1.14) and the thinner legumes were recorded in JAD population (4.75±0.45). ZAJ population showed the higher amounts of seed diameter (3.46±0.35) and ASD population showed the lower amounts (2.45±0.13). The wider rhizomes were observed in SOK population (5.03±0.03) and the thinner rhizomes observed in DED population (3.16±0.01).

Statistical analysis

Different values for the correlation between glycyrrhizic acid content and soil components were observed (Table 6). Based on the results, there are significant correlations between some soil compounds with the content of Glycyrrhizic acid. Glycyrrhizic acid content showed positive correlation with P ($P \leq 0.05$, $R^2 = 0.155$) and negative correlation with pH ($P \leq 0.01$ and $R^2 = 0.348$) and $CaCo_3$ ($P \leq 0.01$ and $R^2 = 0.507$). Glycyrrhizic acid content showed negative correlation with rhizome diameter ($P \leq 0.01$ and $R^2 = 0.222$), average ($P \leq 0.05$, $R^2 = 0.123$) and maximum temperature ($P \leq 0.05$, $R^2 = 0.135$) and positive correlation with annual precipitation ($P \leq 0.01$ and $R^2 = 0.128$) and collecting time ($P \leq 0.01$ and $R^2 = 0.213$). There was no significant correlation between glycyrrhizic acid content and its taxonomy. Taxonomy of *G. glabra* showed positive correlation with altitude ($P \leq 0.01$ and $R^2 = 0.457$) and negative correlation with average ($P \leq 0.01$ and $R^2 = 0.131$) and minimum temperature ($P \leq 0.01$ and $R^2 = 0.202$) (Table 7).

As seen in Table 8, leaf width, legume length, seed and rhizome diameters showed correlation with soil texture ($P \leq 0.01$ and $P \leq 0.05$); leaf width negatively correlated with soil texture ($P \leq 0.05$ and $R^2 = 0.143$), legume length showed correlations with all soil factors ($P \leq 0.01$ and $P \leq 0.05$) and leaf length and seed diameter didn't show any correlation with soil factors. Table 9 represents the correlation between morphometrical data and ecological factors. Based on achieved results, rhizome diameter showed negative correlation with collection date ($P \leq 0.01$ and $R^2 = 0.389$) and annual precipitation ($P \leq 0.01$ and $R^2 = 0.394$). Average ($P \leq 0.05$, $R^2 = 0.123$) and maximum temperature ($P \leq 0.05$, $R^2 = 0.150$) observed positive correlation with rhizome diameter. Width of the leaf and legume showed positive correlation with collection date and annual precipitation ($P \leq 0.01$ and $P \leq 0.05$).

Table 4. Environment data for the localities of collected samples of *G. glabra.*

Loc.	Collecting time	Ann. pptn. (mm)	Av. temp. (°C)	Max temp. (°C)	Min temp. (°C)
AZD	Aug.	31.5	28.4	34.7	22.1
KAL	Aug.	26.25	28.5	35.2	21.9
KSH	Nov.	10.6	13.1	19.1	7.2
HSD	Sep.	29.3	18.1	26.6	9.7
JAD	Jun.	13.6	23.5	31.8	15.2
DED	Sep.	33.25	19.9	27.8	12
ZNJ	Sep.	34.91	19.8	29.0	10.6
SOK	Jun.	10.75	27.8	35.9	19.7
NAQ	Sep.	37.16	20.1	28.3	11.9
MAV	Sep.	39.6	19.9	27.5	12.4
ASD	Jul.	34.6	25.4	35.1	15.7

Note: Loc.: locality, Ann. pptn.: annual precipitation, Av. temp.: average temperature AZD: Azadshahr, KAL: Kalaleh, KSH: Kashan, HSD: Hashtrud, JAD: Jajrood, DED: Dizaj-e Dowl, ZNJ: Zanjan, SOK: Sorkheh, NAQ: Naqadeh, MAV: Mavana, ASD: Asadabad

Table 5. Morphometrical features of different populations of *G. glabra*

Pop.	Leaf length	Leaf width	Legume length	Legume width	Seed diameter	The number of seeds in each legume	Rhizome diameter
AZD	$34.12\pm2.30^{a,b,c}$	$11.65\pm0.81^{c,d}$	$22.92\pm2.68^{b,c}$	$5.76\pm0.18^{b,c}$	2.68 ± 0.36^{c}	4.20 ± 1.09^{b}	3.59 ± 0.01^{e}
KAL	$33.97\pm0.25^{b,c}$	13.95 ± 0.59^{b}	16.09 ± 4.38^{e}	$5.59\pm0.25^{b,c,d}$	2.80 ± 0.27^{c}	2.00 ± 1.44^{c}	3.49 ± 0.01^{f}
KSH	$37.14\pm2.15^{a,b}$	9.61 ± 0.55^{e}	16.05 ± 2.65^{e}	$5.04\pm0.18^{e,d}$	2.72 ± 0.23^{c}	$3.00\pm1.00^{b,c}$	3.58 ± 0.01^{e}
HSD	$31.39\pm3.64^{c,d}$	13.42 ± 1.22^{b}	25.47 ± 2.01^{b}	$6.24\pm0.38^{a,b,c}$	2.87 ± 0.26^{c}	6.60 ± 1.14^{a}	3.64 ± 0.01^{d}
JAD	28.36 ± 1.98^{d}	4.53 ± 1.20^{f}	15.54 ± 4.31^{e}	4.75 ± 0.45^{e}	$2.94\pm0.40^{b,c}$	$3.60\pm0.89^{b,c}$	4.11 ± 0.20^{b}
DED	$30.83\pm1.23^{c,d}$	$12.54\pm1.10^{b,c}$	$17.32\pm4.21^{e,d}$	6.81 ± 0.62^{a}	2.72 ± 0.27^{c}	$3.00\pm1.87^{b,c}$	3.16 ± 0.01^{h}
ZAJ	37.33 ± 1.10^{a}	13.47 ± 1.85^{b}	$21.77\pm3.88^{b,c,d}$	$5.67\pm0.75^{b,c,d}$	3.46 ± 0.35^{a}	$3.40\pm1.81^{a,b}$	4.00 ± 0.19^{c}
SOK	$31.71\pm1.66^{c,d}$	9.96 ± 1.72^{e}	31.29 ± 1.13^{a}	$6.14\pm0.18^{a,b,c}$	2.65 ± 0.25^{c}	6.80 ± 0.837^{a}	5.03 ± 0.03^{a}
NAQ	$36.62\pm1.98^{a,b}$	$13.10\pm1.01^{b,c}$	$19.74\pm3.55^{c,d,e}$	$5.80\pm0.88^{c,b}$	$3.36\pm0.76^{a,b}$	$2.40\pm1.14^{b,c}$	3.29 ± 0.02^{g}
MAV	$33.69\pm1.64^{b,c}$	15.56 ± 1.12^{a}	14.70 ± 1.14^{e}	$5.55\pm0.51^{c,d}$	2.64 ± 0.22^{c}	$3.20\pm0.837^{b,c}$	3.48 ± 0.20^{f}
ASD	$37.12\pm5.19^{a,b}$	$10.68\pm1.05^{d,e}$	$17.74\pm6.90^{e,d}$	$6.29\pm0.57^{a,b}$	2.45 ± 0.13^{c}	$3.60\pm2.30^{b,c}$	3.58 ± 0.01^{e}

Note: Pop.: population, AZD: Azadshahr, KAL: Kalaleh, KSH: Kashan, HSD: Hashtrud, JAD: Jajrood, DED: Dizaj-e Dowl, ZNJ: Zanjan, SOK: Sorkheh, NAQ: Naqadeh, MAV: Mavana, ASD: Asadabad.The descriptive statistics are presented in terms of the mean ± SD. Mean values with the same letters indicate homogeneous subsets for $P \leq 0.05$ according to Duncan test. .^{a,b,c,d,e,f,g,h} Means in the same column with different superscripts differ ($P \leq 0.05$)

Table 6. Correlation between soil compounds and and the amount of glycyrrhizic acid

Parameter	Soil texture	pH	EC	Na	Ca	Cl	K	P	OC	N	$CaCO_3$	Glycyrrhizic acid
Soil texture	1	0.241	0.732**	0.714**	0.608**	0.786**	-0.488**	-0.565**	-0.619**	-0.600**	0.112	-0.171
PH	0.241	1	0.316	0.354*	0.110	0.381*	-0.308	-0.560**	-0.440*	-0.422*	0.817**	-0.590**
EC	0.732**	0.316	1	0.982**	0.943**	0.890**	-0.260	-0.236	-0.568**	-0.436*	0.129	0.043
Na	0.714**	0.354*	0.982**	1	0.894**	0.940**	-0.274	-0.330	-0.636**	-0.514**	0.194	0.005
Ca	0.608**	0.110	0.943**	0.894**	1	0.757**	-0.134	-0.020	-0.409*	-0.282	-0.105	0.298
Cl	0.786**	0.381*	0.890**	0.940**	0.757**	1	-0.369*	-0.584**	-0.779**	-0.715**	0.269	-0.071
K	-0.488**	-0.308	-0.260	-0.274	-0.134	-0.369*	1	0.511**	0.667**	0.684**	-0.006	-0.040
P	-0.565**	-0.560**	-0.236	-0.330	-0.020	-0.584**	0.511**	1	0.771**	0.864**	-0.614**	0.393*
OC	-0.619**	-0.440*	-0.568**	-0.636**	-0.409*	-0.779**	0.667**	0.771**	1	0.972**	-0.379*	0.101
N	-0.600**	-0.422*	-0.436*	-0.514**	-0.282	-0.715**	0.684**	0.864**	0.972**	1	-0.369*	0.108
$CaCo_3$	0.112	0.817**	0.129	0.194	-0.105	0.269	-0.006	-0.614**	-0.379*		1	-0.712**
Glycyrrhizic acid	-0.171	-0.590**	0.043	0.005	0.293	-0.071	-0.040	0.393*	0.101	0.108	-0.712**	1

Note: *Significant difference in α = 5%, ** Significant difference in α = 1%, minus sign shows the negative correlation between data's and plus sign shows positive correlation.

Table 7. Correlation coefficient between climatic conditions, morphometric factors and glycyrrhizic acid

Parameter	Loc.	Alt.	Rhizome diam.	Collecting time	Ann. Pptn.	Av. temp	Max temp	Min temp	Taxonomy	Glycyrrhizic acid
Loc.	1	--0.384*	-0.536**	0.283	0.119	0.013	-0.070	0.096	0.000	0.494**
Alt.	-0.384*	1	0.154	-0.123	0.120	-0.418*	-0.222	-0.598**	0.676**	-0.043
Rhizome diam.	-0.536**	0.154	1	-0.624**	-0.628**	0.351*	0.387*	0.294	0.189	-0.471**
Month	0.283	-0.123	-0.624**	1	0.253	-0.786**	-0.849**	-0.678**	-0.026	0.462**
Ann. Pptn.	0.119	0.120	-0.628**	0.253	1	-0.009	0.066	-0.086	-0.096	0.358**
Av. temp.	0.013	-0.418*	0.351*	-0.786**	-0.009	1	0.974**	0.972**	-0.362**	-0.351*
Max temp.	-0.070	-0.222	0.387*	-0.849**	0.066	0.974**	1	0.894**	-0.262	-0.368*
Min temp.	0.096	-0.598**	0.294	-0.678**	-0.086	0.972**	0.894**	1	-0.449**	-0.316
Taxonomy	0.000	0.676**	0.189	-0.026	-0.096	-0.362**	-0.262	-0.449**	1	0.222
Glycyrrhizic acid	0.494**	-0.043	-0.471**	0.462**	0.358**	-0.351*	-0.368*	-0.316	0.222	1

Note: Loc.: locality, Alt.: Altitude, Ann. Pptn.: annual precipitation, Av. temp: average temperature
*Significant difference in α = 5%, ** Significant difference in α = 1%, minus sign shows the negative correlation between data and plus sign shows positive correlation.

Table 8. Correlation coefficient between some morphometry elements and soil factors

Factors	Texture	pH	Ec	Na	Ca	Cl	K	P	Oc	N	$CaCo_3$	Taxonomy
Leaf length	-0.120	-0.121	-0.168	-0.177	-0.017	-0.170	-0.082	-0.015	-0.005	-0.085	-0.198	0.044
Leaf width	-0.378*	-0.299	-0.012	0.020	0.119	-0.177	0.144	0.509**	0.175	0.266	-0.358*	-0.275
Legume length	0.553**	0.470**	0.693**	0.724**	0.613**	0.727**	-0.128	-0.412*	-0.585**	-0.505**	0.358*	-0.187
Legume width	0.099	0.010	0.332	0.385*	0.380*	0.288	-0.302	0.103	-0.281	-0.215	-0.313	-0.166
Seed	0.373*	0.538**	0.470**	0.561**	0.272	0.619**	-0.223	-0.546**	-0.577**	-0.519**	0.566**	-0.285
Seed diameter	-0.184	-0.120	-0.150	-0.186	-0.150	-0.114	0.181	-0.019	0.048	0.044	0.159	0.255
Rhizome diam.	0.748**	0.383*	0.611**	0.617**	0.447**	0.725**	-0.043	-0.621**	-0.586**	-0.553**	0.538**	0.189

Note: Pearson's correlation coefficient is indicated with level of significance ($P \leq 0.05$ and $P \leq 0.01$). Negative and positive correlation between factors are shown by minus and plus sign. **Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed)

Table 9. Correlation coefficient between some morphometry elements and soil factors

Factors	Alt.	Collecting time	Ann. pptn.	Av. Temp.	Min temp.	Max temp.
Rhizome diameter	0.154	-0.624**	-0.628**	0.351*	0294	0.387*
Leaf length	-0.064	0.337	0.272	-0.134	-0.138	-0.124
Leaf width	-0.136	0.511**	-0.744**	-0.141	-0.132	-0.139
Legum length	-0.233	-0.241	-0.313	0.270	0.271	0.252
Legum width	-0.131	0.388*	-0.477**	-0.057	-0.064	-0.045
Seed	0.025	-0.190	-0.329	0.038	0.025	0.050
Seed diameter	0.343	-0.243	-0.227	-0.154	-0.209	-0.095

Note: Alt: Altitude, Ann. pptn: annual precipitation, Av. temp: average temperature. Pearson's correlation coefficient is indicated with level of significance ($P \leq 0.05$ and $P \leq 0.01$). Negative and positive correlation between factors are shown by minus and plus sign. **Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed)

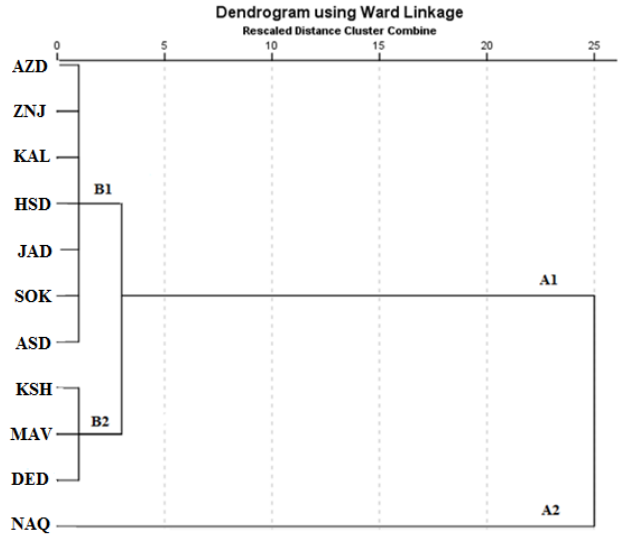

Figure 4. Dendrogram obtained by the hierarchical cluster analysis of the amounts of glycyrrhizic acid and morphometrical data.

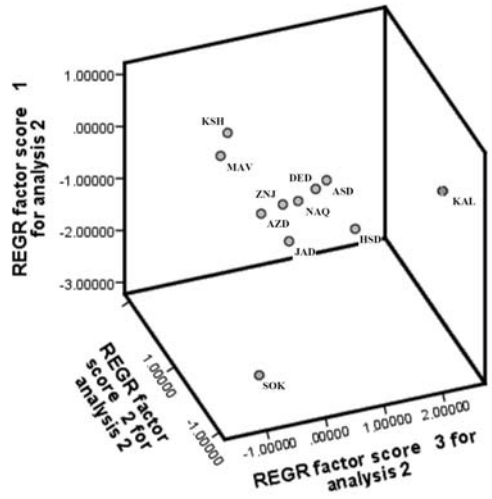

Figure 5. Principal component analysis (PCA) of the morphometrical data and amounts of glycyrrhizic acid. Abbreviation of the names are described in Table 1.

Table 10. Component matrix of principal components analysis (PCA) of the morphometry and amount of glycyrrhizic acid from different populations of *G. glabra*

Factors	Component		
	1	2	3
Leaf width	0.790	0.319	-0.320
Leaf length	0.730	0.049	0.457
Legum width	0.690	0.651	-0.181
Glycyrrhizic acid	0.610	-0.221	0.539
Legum length	-0.288	0.883	0.232
The number of seeds in each legume	-0.487	0.832	-0.055
Rhizome diameter	-0. 713	0.508	0.327
Seed diameter	-0.726	-0.577	0.015

The dendrogram achieved from Hierarchical cluster analysis (HCA) of the relative contents of the glycyrrhizic acid and morphometrical data (Figure 4) showed that 11 populations of *G. glabra* were divided into two main clusters in average distance value (ADV) of 25: cluster A2 including NAQ and cluster A1, which is then divided into two sub-clusters at ADV 2. These are: sub-cluster B2 including KAS, MAV, DED and sub-cluster B1 including other populations.

The graph obtained from principal components analysis (PCA) of the morphometry and amount of glycyrrhizic acid (Figure 5) indicated that 11 populations of *G. glabra* were placed into three groups; group 1 including NAQ, group 2 containing JAD and group 3 including other populations. Based on Table 10, leaf dimension are effective factors in separating different populations of *G. glabra*.

Discussion

The amount of secondary metabolites from the roots of licorice depended on the growing conditions (Zhang et al. 2011). Analyses of populations belonging to two varieties of *Glycyrrhiza glabra*, collected from 11 locations in Iran showed different amounts of Glycyrrhizic acid. In *G. glabra* var. *glabra* the highest content of glycyrrhizic acid was from AZD population and HSD population showed the lowest amount. In *G. glabra* var. *glandulifera* the lowest and highest content of glycyrrhizic acid were recorded in KSH and NAQ populations, respectively. The amount of glycyrrhizic acid for SOK, JAD, KAL and AZD showed similar amounts, may be related to the similar environment they have. Fenwick et al. (1990) showed the amount of glycyrrhizic acid was related to the varieties, location and collecting time. In contrast, we observed no correlation between the amount of glycyrrhizic acid and taxonomy. The amounts of glycyrrhizic acid was correlated with location and collecting time in our study. The amount of glycyrrhizic acid contents showed positive correlation with P and negative correlation with pH and $CaCO_3$ (Table 6). In fact, the amount of glycyrrhizic acid is decreasing with higher pH,. Zhang et al. (2011) showed that the amount of glycyrrhizic acid in *Glycyrrhiza uralensis* in north China was closely related to the amount of soil P and K, but soil pH showed no significant correlation. According to Hosseini et al. (2014) and Oloumi and Hassibi (2011), there were no significant correlation between the amount of Glycyrrhic acid and edaphic factors. We had a broader geographical sampling in this study.

Based on our results, the amount of glycyrrhizic acid content is negatively related with maximum and average temperature and represented positive relation with annual precipitation. Similarly, high precipitation and low temperature (maximum and average) resulted in higher amount of glycyrrhizic acid content. Hosseini et al. (2014) and Kriker et al. (2013) represented that temperature and precipitation were effective factors on glycyrrhizic acid content. Similar to our results, positive correlation between annual precipitation and glycyrrhizic acid content in *G. uralensis* was reported from China (Zhang et al. 2011).

According to the morphometrical analysis, thinner rhizomes from Glycyrrhiza glabra, showed higher amount of glycyrrhizic acid contents (Table 7). Similar results achieved by Usai et al. (1995) and Bolouri Moghaddam et al. (2009). On the other hand, annual precipitation showed negative correlation with rhizome diameter. In rhizomes with higher diameter, cortex and epiderm were replaced with cork tissue. Whereas the glycyrrhizic acid is produced in parenchyma tissues. Increasing in rhizome diameter therefore results in decreasing parenchyma tissue (Bolouri Moghaddam et al. 2009).

Our results indicated that *G. glabra* var. *glabra* is found in locations with lower altitude and higher temperature. And conversely *G. glabra* var. *glandulifera* is founds in regions with higher altitude and lower temperature. According to the Figure 4 and 5, NAQ population separated from other populations because of the highest amount of glycyrrhizic acid contents. Not only glycyrrhizic acid contents, but also PCA and HCA could not separate two varieties of *G. glabra*. In Figure 4 (PCA) papulations divided to 2 main clusters (A1, A2) that this division has been based on the amount of Glycyrrhizic acid. The second cluster (A2) was divided into two clusters that could not separate two varieties, although MAV and DED showed similar ecological factors and other populations of different varieties were grouped together. These results showed that morphometrical factors and the amount of glycyrrhizic acid could not separate two varieties. Analyses of the chemical components and morphometrical factors released same results and could not separate two varieties.

It seems that the contents of glycyrrhizic acid depend on the soil composition and the environmental factors. The amount of glycyrrhizic acid content was increased in the soils with lower pH, more P and $CaCo_3$. Collecting time and temperature also affected the content of Glycyrrhizic acid. Higher temperature positively affected the amount of glycyrrhizic acid content. The plants with thinner rhizomes had higher amount of glycyrrhizic acid content. In addition, there is no correlation between the taxonomy and the amount of glycyrrhizic acid content.

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