J. bio-sci. 22: 59-67, 2014 **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index

INFRA-SPECIFIC MORPHOLOGICAL DIVERSITY IN *PHLOMIS OLIVIERI* (LABIATAE)

Seyed Mehdi Talebi [*]

Department of Biology, Faculty of Sciences, Arak University, Arak38156-8-8349, Iran

Abstract

Context: Compare the effect of different ecological conditions on phenotypical traits of *Phlomis olivieri*.

Objective: To observe morphological variation between and within different populations of *Phlomis olivieri* Benth.

Materials and Methods: Morphological characters of eleven populations of *Phlomis olivieri* were investigated in the both levels: between and within populations. Samples were collected from different parts of Iran and from each population, 3 to 4 samples were elected randomly and sixteen quantitative and qualitative morphological traits from the both vegetative and reproductive organs were examined.

Results: Analysis of variance test as well as one-sample test confirmed significant variations for quantitative morphological traits. Morphological features varied between populations, consequently populations were separated from each other in the UPGMA tree and also PCO and PCA plots, among these, populations K, A, D and B placed far from others. Some degrees of polymorphism in morphological traits occurred within populations and individuals of each stand separated from others.

Conclusion: This study showed that ecological factors had strong effect on morphological features of different populations of same plant species. This phenomenon was called phenotypic plasticity which created infraspecific variations at both levels interpopulation and intrapopulation or morphological polymorphism among populations.

Key words: Ecology, populations, morphological characteristics, polymorphism, UPGMA, *Phlomis olivieri*.

Introduction

The genus *Phlomis* L.*,* of the family Lamiaceae, consists of about 100 species (Kyriakopoulou *et al.* 2001, Albaladejo *et al.* 2004), which nineteen of them naturally occurred in Iran (Jamzad 2012). These species were explained by Dioscorides as medicinal herbs, and were in practice ethno- pharmacologically in herbal drugs for respiratory tract ailments and for local healing of injuries. In addition, some species of *Phlomis* were used in folk medicine for their analgesic and antidiarrheal properties, and for the treatment of ulcers and hemorrhoids. In some studies, different activities such as anti-inflammatory, immunosuppressive, antimutagenic, antinociceptive, antifibriel, free radical scavenging, antimalarial as well as antimicrobial effects have seen in members of *Phlomis* genus (Sarkhail *et al.* 2006).

Fundamental design characteristics in plant, i.e. structure and architecture, at both the cellular and whole levels, are directly influenced by the immediate environment in terms of resource supply and biotic or abiotic stress. Plants can respond to stress over evolutionary time by adaptation phenomenon, but can also respond to temporal and spatial fluctuations in external stresses through adjustment in their shape and structure (phenotypic plasticity), allowing them to counterbalance the effects of the stress over their life span (Read & Stokes 2006). For example, self-supporting terrestrial plants can be exposed to a range of changing stresses, such as wind (Sellier & Fourcaud 2005), avalanches (Johnson 1987) and other soil mass movements.

[*] Corresponding author *E-mail:* seyedmehdi_talebi@yahoo.com

Various populations of the same species often present in a range of ecological conditions, encompassing variation in moisture levels, soil composition, growing season's length, animal-pollinated species, even variation in the composition of the pollinators. The mentioned differences in environmental conditions may create different selection pressures across the species range, resulting in genetic divergence among populations (Briggs & Walters 1984).

Phlomis olivieri Benth. is a members of the genus *Phlomis* and grows naturally in different regions of northern, north-western, western and central Iran (Jamzad 2012). In order to compare the effect of different environmental factors on phenotypical traits of *Phlomis olivieri*, in present study, morphological features of eleven populations of this species were examined between and within populations.

Material and Methods

In the present study, eleven natural populations of *Phlomis olivieri* were collected from different regions of Iran (Table 1) during spring 2011-2012. Samples were identified on the basis of descriptions provided in Flora Iranica and Flora of Iran (Rechinger 1982, Jamzad 2012). From each population 3 to 4 samples were elected randomly and sixteen quantitative and qualitative morphological traits from the both vegetative and reproductive organs such as stem height and its number of branches , shape, length and width of basal and floral leaves and their pedicle length, calyx and corolla dimensions were compared between as well as within populations.

Table 1. Locality address of the studied populations of *Phlomis olivieri.*

Habitat address	Population
Markazi province, Zarandiyeh, 1330 m.	A
Markazi province, Shahsavan Kandi, 1270 m.	B
Tehran province, Sohanak, 1900 m.	C
Zanjan province, Saein Qaleh, 1542 m .	D
Zanjan province, Mianeh, 1680 m.	E
Kurdistan province, Sanandaj, Abidar mountain, 1450 m.	F
Qazvin province, Avaj, 1600 m.	G
Ilam province, near Seimareh River, 1000 m.	H
Ilam province, west of Ilam,875 m.	I
Kermanshah province, 1892 m.	J
Markazi province, Tafresh, 2243 m.	K

The means, std. deviations and also variance of morphological traits were determined for populations. For grouping of populations and individuals, data were standardizes (mean = 0, variance = 1) and used for multivariate analyses including UPGMA (Unweighted Paired Group using Average method), and Principal Coordinate Analysis (PCO) (Podani 2000). One-way ANOVA test was employed assess significant difference for quantitative characteristics between populations. Pearson correlations coefficients were used among quantitative morphological traits to show relationship between them. MVSP ver.3.1 (2004) and SPSS ver. 9 (1998) software's were used in the statistical analyses.

Results

In the present study phenotype plasticity of eleven populations of *Ph. olivieri* were investigated. For this purpose, sixteen quantitative and qualitative morphological features from the both vegetative and reproductive organs examined in the two levels between (inter) and within (intra) populations (Table 2).

Space for Table 2 to be inserted from
File name: Table 2 of P-9-Phlomis paper

Table 3. ANOVA analyses of quantitative the morphological characteristics.

Characters		Sum of Squares	df	Mean Square	F	Sig.
Basal leaf length	Between groups	131.614	10	13.161	8.412	0.000
	Within groups	40.678	26	1.565		
	Total	172.292	36			
Basal leaf width	Between groups	11.589	10	1.159	6.825	0.000
	Within groups	4.415	26	0.170		
	Total	16.004	36			
Number of Flowers	Between groups	17.703	10	1.770	15.342	0.000
	Within groups	3.000	26	0.115		
	Total	20.703	36			
Calyx length	Between groups	200.516	10	20.052	5.324	0.000
	Within groups	97.917	26	3.766		
	Total	298.432	36			
Calyx width	Between groups	49.463	10	4.946	9.118	0.000
	Within groups	14.104	26	0.542		
	Total	63.568	36			
Petal length	Between groups	774.187	10	77.419	22.852	0.000
	Within groups	88.083	26	3.388		
	Total	862.270	36			
Floral leaf length	Between groups	11081.194	10	1108.119	47.714	0.000
	Within groups	603.833	26	23.224		
	Total	11685.027	36			
Floral leaf width	Between groups	548.561	10	54.856	14.818	0.000
	Within groups	96.250	26	3.702		
	Total	644.811	36			

Interpopulation study:

The studied populations were selected from different regions of Iran; therefore morphological features of individuals varied between populations. Analysis of variance (ANOVA) showed significant variations for some quantitative morphological characteristics such as: stem height, basal leaf width, basal leaf length, floral leaf length, floral leaf width, number of flowers, calyx length, calyx width, calyx length/width ratio and petal length (Table 3). But one-sample test showed significant difference ($p<0.01$) for all the examined quantitative morphological features (Table 4). In addition, qualitative characters such as basal and floral leaves shapes differed between populations. Basal leaves shapes were presented as: linear, lanceolate, linear- lanceolate, ovate and oblong. These conditions were true about floral leaves shapes, and different shapes such as: lanceolate, linear, linear- lanceolate, oblong and also ovate were observed. Box and whisker plots showed that numbers of morphological characteristics such as basal and floral leaf length/width ratio had normal ranges of distribution, but some of them such as branch number were not normally distributed domain. Range of changes in the numbers of traits, for example, number of flowers of each inflorescent cycle, calyx leaf/ width ratio and pedicle in some populations varied so much that the characters were out of ranges (Fig.1).

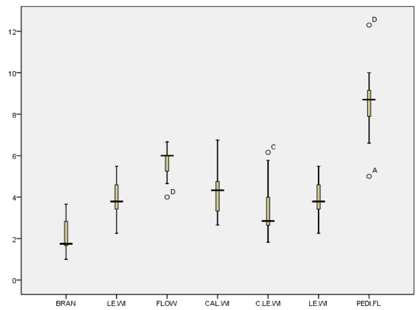

Fig.1. Box and Whisker plot of some quantitative morphological traits.

Descriptive statistical analysis of quantitative characters showed that maximum variances as well as standard deviations were seen in floral leaf length, stem height and petal length. In contrast, floral leaf pedicle, basal leaf width and flower no. have minimum variances and standard deviations (Table 5). The studied populations were separated from each others in the UPGMA tree (Fig. 2) and also PCA and PCO plots (Figs. 3, 4) of morphological characteristics. The arrangements of populations in the mentioned diagrams were similar and populations K, A, D and B were placed far from others.

Table 4. One-sample test of the quantitative morphological traits.

	Test Value = 0					
					95% Confidence Interval of the difference	
Characters	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Stem height	31.696	36	0.000	34.92973	32.6947	37.1647
Number of branches	9.498	36	0.000	2.16216	1.7005	2.6238
Basal leaf length	9.048	36	0.000	3.25405	2.5246	3.9835
Basal leaf width	8.802	36	0.000	0.96486	0.7426	1.1872
leaf length/width	16.409	36	0.000	3.89000	3.4092	4.3708
Basal pedicle	7.970	36	0.000	1.67703	1.2503	2.1038
Number of flowers	45.092	36	0.000	5.62162	5.3688	5.8745
Calyx length	28.207	36	0.000	13.35135	12.3914	14.3113
Calyx width	20.104	36	0.000	4.39189	3.9488	4.8349
Calyx leaf/width	12.762	36	0.000	3.37973	2.8427	3.9168
Petal length	22.103	36	0.000	17.78378	16.1520	19.4155
Floral leaf length	8.386	36	0.000	24.83784	18.8309	30.8447
Floral leaf width	9.711	36	0.000	6.75676	5.3457	8.1678
Floral pedicle	14.642	36	.000	0.85405	0.7358	0.9724

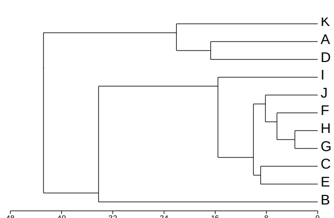

Fig. 2. Morphological UPGMA tree of the studied populations.

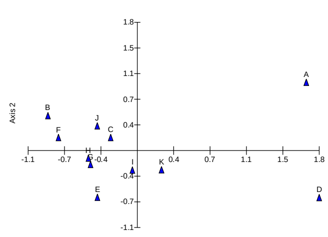

Fig. 3. Morphological PCA plot of the studied populations.

Table 5. Descriptive statistical analysis of the studied morphological features.

Characteristics	Minimum	Maximum	Mean	Std. Deviation	Variance
Stem height	18.00	52.00	34.9297	6.70331	44.934
Number of branches	0.00	6.00	2.1622	1.38471	1.917
Basal leaf length	1.50	12.50	3.2541	2.18767	4.786
Basal leaf width	0.30	2.80	0.9649	0.66676	0.445
leaf length/width	1.08	7.33	3.8900	1.44197	2.079
Basal pedicle	0.00	5.80	1.6770	1.27990	1.638
Number of flowers	4.00	7.00	5.6216	0.75834	0.575
Calyx length	10.00	20.00	13.3514	2.87920	8.290
Calyx width	2.00	8.00	4.3919	1.32882	1.766
Calyx leaf. width	1.57	10.00	3.3797	1.61083	2.595
Petal length	11.0	28.0	17.78	4.894	23.952
Floral leaf length	8.00	65.00	24.8378	18.01622	324.584
Floral leaf width	3.00	17.00	6.7568	4.23219	17.911
Floral pedicle	0.30	2.10	0.8541	0.35480	0.126

Intrapopulation study

In order to compare intrapopulation variations (polymorphism) between the studied population's members of this species, of each population 3-4 individuals were elected randomly and their morphological traits were examined carefully. Morphological characteristics varied between individuals and therefore morphological polymorphism was seen within populations. These variations were seen in the both qualitative and quantitative features. Analyses of correlations showed significant positive/ negative correlations between traits. For example, stem height had a significant negative correlation ($p<0.05$, $r= -0.39$) with branch number, but a significant positive correlation ($p<0.01$, $r= 0.42$) was seen between stem height with pedicle of floral leaf. Basal leaf length had significant positive correlations ($p<0.01$) with basal leaf width, pedicle length, corolla length as well as floral leaf dimensions. But this features had significant negative correlations ($p<0.05$) with calyx length and flower number per each inflorescence cycle. Furthermore, flower number per each inflorescence cycle had a significant positive correlation ($p<0.05$, $r= 0.39$) with calyx width, in contrast significant negative correlations ($p<0.01$) found between the mentioned trait with basal leaf width and its pedicle length, floral leaf width and length.

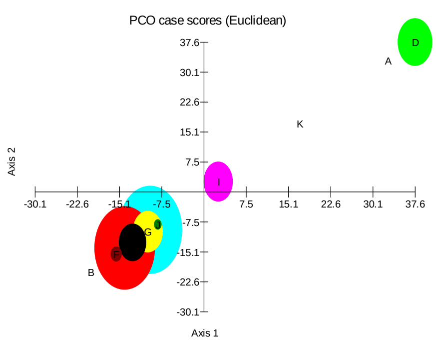

Fig. 4. PCO plot of morphological characters of the studied populations.

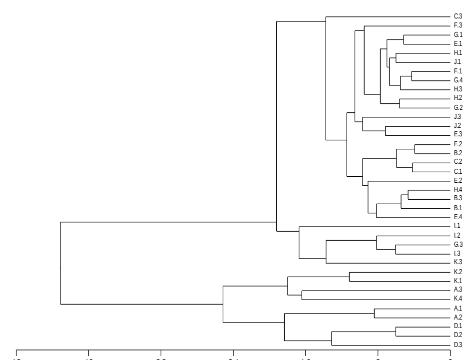

Fig. 5. UPGMA tree of individuals of the studied populations.

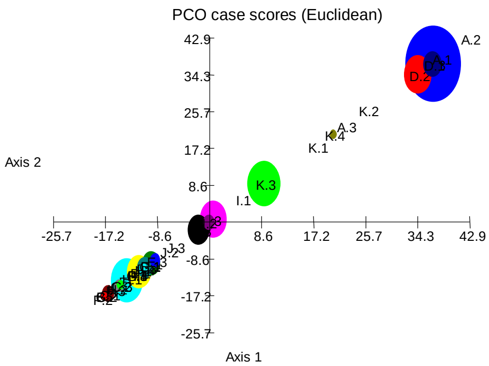

Fig. 6. PCO plot of the studied population's individuals.

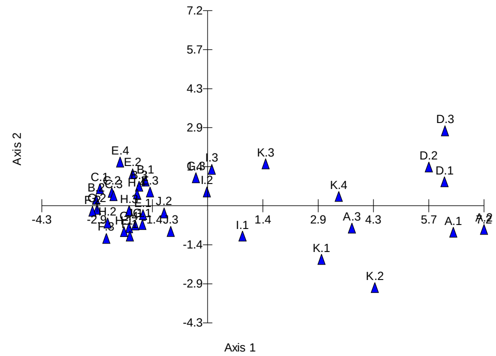

Fig. 7. PCA plot of the studied population's individuals.

Individuals of the studied populations were separated from others in the UPGMA tree (Fig. 5) as well as PCO and PCA plots (Figs. 6, 7). High morphological polymorphism existed in some population such as populations C, G and E. For example populations G and E consisted of four individuals and these samples placed far from others and arranged separately. These conditions were true about population C, which two of three individuals, namely C_1 and C_2, were closely related to each other, but individual C_3 placed far from others. In contrast members of population D arranged associated, in addition samples of populations I placed near each other's and therefore lowest morphological polymorphism were found between them.

Discussion

Species of the genus *Phlomis* had a wide distribution from China through Eurasia to the Mediterranean. Two centers of diversity can be recognized, south and east Anatolia to north- west of Iran, and central Asian parts of the old Soviet to east of China (Azizian & Moore 1982). Because of the importance of Iran as one of the main diversity center of this genus, in the present study, in order to examination the effects of different ecological factors or on the other hand different environmental conditions, in creation of intraspecific variations, thirty seven individuals from eleven populations of *Phlomis olivieri* were investigated phenotypically. Morphological traits have important value in taxonomy of the genus *Phlomis* and are used as main characters in infrageneric classification and also different studies confirmed it. For instance, Moench (1794) recognized morphological differences within *Phlomis,* which he believed that these characteristic enough to split the *Phlomis* into two separate genera, *Phlomis* and *Phlomoides*. These features have also been recognized by many other authors (Bentham 1832-1836, Kamelin & Makhmedov 1990a,b) and the discussion since has been to which level in the taxonomic hierarchy the two *Phlomis* groups should be assigned. This division has been based on several traits, including habit (the *Phlomis* group are shrubs or sub-shrubs, while members of the group *Phlomoides* are herbaceous), certain leaf characteristics, the shape and colour of the corolla and strong cytological data (Azizian & Moore 1982a, Azizian & Cutler 1982b, His-wen & Hedge 1994). Morphological features varied between populations. These variations occurred in the both qualitative characteristics such as floral and basal leaves shapes and also quantitative features of vegetative and reproductive organs.

Leaf quantitative characteristics are of main traits in plants, which varied in different ecological conditions and this subject is very important for plant taxonomy. These traits differed between populations and ANOVA test confirmed it. In plants subjected to recurring mechanical stresses such as wind loading or other mechanical perturbations, several responses are observed. Leaf number and area may diminish (Stokes *et al.* 1995, Niklas 1996) which reduces wind induced drag on the crown (Sellier & Fourcaud 2005), but also reduces photosynthesis. In addition, features of reproductive organs such as calyx and corolla dimensions differed between samples. Micro- evolutionary investigations of intraspecific variation in floral traits are important for identifying the role of adaptive evolution in floral diversification. Placing these studies in a spatial context can elucidate the genetic and ecological factors underlying evolutionary modifications to floral traits and mating strategies (Herrera *et al.* 2006). The patterns of population's arrangement in the plots and trees marked high variations and similarities between different morphological characteristics of populations. These resemblance or difference in phenotypical traits could be consequent of habitat effect. For example, populations K, A and D placed far from others in a distinct group and among these, population K was seen separately. The reason of it may be related to habitat altitude. Because population K found in highest habitat and at least ca. 330 m difference in altitude was seen with other populations. This condition was true about population I and this population arranged separately. The mentioned population found in lowest habitat and this subject influenced morphological traits of plants. It is very important to know that variation within species reflects adaptations to specific environmental conditions (Darwin 1859, Clements 1908, Turesson 1922). Morphological traits of fifteen geographical populations of *Stachys inflata* benth. as well as five ecological factors of their habitat were examined. The arrangements of populations in the morphological and ecological trees and plots were similar. Therefore, between different populations, similarity or dissimilarity in habitat's ecological features can cause sameness or difference in the morphological characteristics of plants. This subject confirmed effect of ecological and phytogeographical factors on phenotypic plasticity of plants (Talebi *et al*. 2014).

Ph. Olivieri has a wide range of habitat and grows in different regions of Iran (Rechinger 1982, Jamzad 2012). This disturbance may be linked to phenotypical variation which occurred in this species, because, without morphological plasticity, any species cannot present in different habitats and adapt with various ecological factors that present on it.

Relationship between ecological variation and phenotypic difference within species showed that species grows in a wider range of habitats, or in the other words habitat generalists, will be more variable in phenotypic characteristics than species that occupy a narrow range of habitats or habitat specialists (Valen 1965, Baker 1974).

Furthermore, morphological traits showed polymorphism and varied among populations. From each population, 3-4 individuals were collected randomly and performed multivariate analyses confirmed these variations and sample of studied populations placed separately. Results showed that the highest polymorphism occurred in populations C, G and E and members of these populations placed separately far from each others. The occurrence of intra-population variation (polymorphism) is very important, because it related to sympatric speciation. Evolutionary biologists are particularly attentive to intra-population variation, because of its implications for speciation. Many of the candidate cases for sympatric phases of speciation involve individual specialization, and the establishment of a resource polymorphism (Via 2001). In the early stages of speciation, individual specialization may lead to a niche expansion, resulting in reduced intra specific competition (Valen 1965). Individual specialization is at the interface between community and evolutionary ecology, and yet there are few methods available for its detection or accurate description (Bolnick *et al*. 2002).

References

Albaladejo R G, Aparicio A, Silvestre S. 2004. Variation patterns in the *Phlomis* × *composita* (Lamiaceae) hybrid complex in the Iberian Peninsula. *Bot J Linn So.* 45, 97–108. http://dx.doi.org/10.1111/j.1095-8339.2003.00266.x

Azizian D, Cutler D F. 1982 Anatomical, cytological and phytochemical studies on *Phlomis* L., and *Eremostachys* Bunge (Labiatae). *Bot J Linn Soc* 85, 249-281.http://dx.doi.org/10.1111/j.1095-8339.1982.tb00373.x

Azizian D, Moore D M. 1982. Morphological and palynological studies in *Phlomis* L., *Eromostachys* Bunge and *Paraphlomis* Prain (*Labiatae*). *Bot J Linn Soc* 85, 225-248. http://dx.doi.org/10.1111/j.1095-8339.1982.tb00373.x

Baker H G.1974. The evolution of weeds. *Annu Rev Ecol Syst* 5, 1-24. http://dx.doi.org/10.1146/annurev.es.05.110174.000245

Bentham G. 1832-1836. *Labiatarum Genera et Species*. James Ridgeway and Sons. London, UK.

Bolnick D, Yang L, Fordyce J, Davis J, Svanback R. 2002. Measuring individual-level resource specialization. *Ecology* 83, 2936–2941. http://dx.doi.org/10.1890/0012-9658(2002)083[2936:MILRS]2.0.CO;2

Clements F E. (1908) An ecologic view of the species conception. *Am Nat* 42, 253-281. http://dx.doi.org/10.1086/278930

Darwin C. 1859. *On the Origins of Species by Natural Selection, or the Preservation of Favored Races in the Struggle for Life*, Cambridge, MA, Harvard University Press.

Herrera C M, Castellanos M C, Medrano M. 2006. Geographical context of fl oral evolution: Towards an improved research programme in floral diversification. In*:* Harder L D, Barrett SCH, *Ecology and evolution of flowers*. Oxford University Press, Oxford, UK, 278–294.

Hsi-Wen L, Hedge I C. 1994. *Phlomis* Linnaeus. In*:* Raven P H, Wu, and Zhengyi, eds., *Flora of China*. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 143-155.

Jamzad Z. 2012. *Flora of Iran, no 76, Lamiaceae*, Ministry of Jihad-e- Agriculture, Research Institute of Forests & Rangelands Press ,Iran.

Johnson E A. 1987. The relative importance of snow avalanche disturbance and thinning on canopy plant populations. *Ecology* 68, 43–53. http://dx.doi.org/10.2307/1938803

Kamelin R V, Makhmedov A M. 1990. A new system of the genus *Phlomis* (Lamiaceae). *Botaniceskij Zurnal* 75, 1163-1167.

Kamelin R V, Makhmedov A M. 1990. The system of the genus *Phlomoides* (Lamiaceae). *Botaniceskij Zurnal* 75, 241-250.

Kyriakopoulou I, Magiatis P, Skaltsounis A L, Aligiannis N, Harvala C A..2001. New phenylethanoid glycoside with free-radical scavenging and antimicrobial activities from *Phlomis samia*. *J Nat Prod* 64 (8), 1095–109. http://dx.doi.org/10.1021/np010128+, PMid:11520237

Moench C. 1794. *Methodus Plantas Horti Botanici et Agri Marburgensis, a straminum situ describendi*. Marburgi Cattorum, Marburg, Germany. http://dx.doi.org/10.5962/bhl.title.304

Niklas K J. 1996. Differences between *Acer saccharum* leaves from open and wind-protected sites*. Ann Bot -London* 78, 61-66. http://dx.doi.org/10.1006/anbo.1996.0096

Podani J. 2000. *Introduction to the Exploration of Multivariate Biological Data*. Backhuys Publishers, Leiden.

Read J, Stokes A. 2006. Plant biomechanics in an ecological context. *Am J Bot* 93(10), 1546-1565. http://dx.doi.org/10.3732/ajb.93.10.1546

Rechinger K H. 1982. *Phlomis*, Flora Iranica, No.150: Akademische Druck-u. Verlagsanstalt, Graz, Austria. 292-316.

Sarkhail P, Monsef E H R, Amin G, Surmaghi M H S, Shafiee A. 2006. Phytochemical study of *Phlomis olivieri* Benth. and *Phlomis persica* Boiss *Daru* 14, 115-121.

Sellier D, Fourcaud T. 2005. A mechanical analysis of the relationship between free oscillations of *Pinus pinaster* Ait. saplings and their aerial architecture. *J Exp Bot* 56, 1563–1573. http://dx.doi.org/10.1093/jxb/eri151, PMid: 15837709

Stokes A, Fitter A H, Coutts M P. 1995. Responses of young trees to wind and shading: effects on root architecture. *J Exp Bot* 46, 1139-1146. http://dx.doi.org/10.1093/jxb/46.9.1139

Talebi S M, Salahi Esfahani G, Azizi N. 2014b. Inter and intrapopulation variations in *Stachys inflata* Benth. Based on phenotype plasticity (an ecological and phytogeographical review). *Int Res J Biol Sci* 3 (2), 9- 20.

Turesson G. 1922. The genotypical response of the plant species to the habitat. *Hereditas* 3, 211–350. http://dx.doi.org/10.1111/j.1601-5223.1922.tb02734.x

Van Valen L. 1965. Morphological variation and width of ecological niche. *Am Nat* 99, 377-390. http://dx.doi.org/10.1086/282379

Via S. 2001. Sympatric speciation in animals: the ugly duckling grows up. *Trends Ecol Evol.16*, 381–390. http://dx.doi.org/10.1016/S0169-5347(01)02188-7