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Inter and Intrapopulation variations in *Stachys inflata* Benth. based on Phenotype plasticity (An Ecological and Phytogeographical Review)

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Abstract

In present study, inter and intra-population variations of Stachys inflata were investigated. For this purpose fifteen geographical populations of this species were collected from different parts of Iran and for each habitat five ecological factors were examined. Totally, twenty two quantitative and qualitative morphological characters were examined from both vegetative and reproductive organs. ANOVA test and also one-sample T-test showed significant difference for some quantitative characters. In intrapopulation section, 3-4 individuals were elected randomly for each population and totally 59 individuals were studied. Individuals were separated from others in PCO and PCA plots and also average linkage tree. Significant correlations were found between some of quantitative morphological characters with studied ecological factors of habitat. In each population, average amounts of quantitative morphological character were used for interpopulation study. Variations in morphological characters were seen between populations and they were separated in PCO and PCA plots and also UPGMA tree. These populations belong two different phytogeographical subprovinces and also ecological factors were different between habitats; therefore habitats were separated in trees and plots. The arrangements of populations in morphological and ecological trees and plots were similar. Therfore between different populations, similarity or dissimilarity in habitat's ecological features can cause sameness or difference in morphological characters of plants. This subject confirmed effect of ecological and phytogeographical factors on phenotypic plasticity of plants.

Keywords: ecology, *Stachys inflata,* phenotype plasticity, populations.

Introduction

Physiological, morphological and genetically variations were seen in populations of species that occurred in different habitat, these variations were created in response to contrasting environmental conditions. In many plant species, studies of population on the pattern of variation have showed the occurrence of localized populations each adapted to the particular ecological conditions of their habitat[1-5].

Ecological conditions of habitat reflect the traits of an individual because they are fitted to environment condition[6]. For the reason that the similarities between individuals in a population are enough great to be considered as one species, it is expected that the total difference in characters (for example, morphology, behavior, genetic material, and physiology) among those individuals is therefore much lower than in a sample of the same number of individuals in a different species. Against this, considerable diversity can occurrence among or within populations of the same species. The mentioned variations could be the result of phenotypic, genotypic or physiologic response to a particular condition of environment. These variations are named phenotypic plasticity. Creations of multiple phenotypes from a single genotype, related to environmental conditions are defined as phenotypic plasticity[7]. Resent study confirmed that a wide diversity that present in different organism showed phenotypic plasticity in reply to biotic and abiotic aspects of their habitats[8,9].

The genus *Stachys* L. is belong to Labiatae family and has 38 species in Iran which classified into two subgenus. *Stachys inflata* Benth. is one of them which placed in subgenus *Stachys*[10]. This species is Irano-Turanian element which widely distributed in different regions of the country. In order to study the effect of different ecological factors on phenotype plasticity in this species, in present study morphological characters of fifteen geographical populations of *st. inflata* were investigated at inter and intrapopulation levels.

Material and Methods

In this study, fifteen geographical populations of *Stachys inflata* Benth. were collected from different regions of Iran (table-1) during spring 2012-2013. Samples were identified based on descriptions provided in Flora Iranica[11] and Flora of Iran[10]. From each population, 3 to 4 samples were elected randomly and twenty two quantitative and qualitative morphological characters from both vegetative and reproductive organs such as stem height and its branches number, length and width of basal and floral leaves and also calyx and corolla dimensions were examined between and within populations.

Table-1
Habitat address of the studied populations

Populations	Habitat
Abas Abad	Markazi province, Zarandiyeh, Kharaghan, Abas Abad,1900m
Ghargh Abad	Markazi province, Saveh, Ghargh Abad, 1461m
Joshaghan	Markazi province, Zarandiyeh, Kharaghan, Joshaghan,1954m
Vardeh	Markazi province, Zarandiyeh, Vardeh,1455m
Vidar	Markazi province, Zarandiyeh, Kharaghan, Vidar,1657m
Abegarm	Markazi province,Mahallat, Abegarm,1737m
Nimvar	Markazi province,Mahallat, Nimvar,1523m
Jasb	Markazi province,Delijan, Jasb,1499m
Ivanaki	Semnan province, Ivanaki, 1107m
J. Boein	Markazi province, Zarandiyeh to Boein Zahra road, 1516m
Salafchegan	Qum province, Salafchegan, 1417m
Rangraz	Markazi province, Zarandiyeh,Rangraz,1393m
Haraz	Tehran province, Haraz road, 2130m
Peyghambar	Markazi province, Zarandiyeh, Peyghambar,1431m
Nobaran	Markazi province, Saveh, Nobaran,1620m

In order to study the effect of different ecological factors on morphological characters of *St. inflata* populations, five ecological factors such as: longitude (E°), latitude (N°), elevation (in meter), average of minimum and maximum temperature (in C°) were examined. Longitude, latitude and elevation were calculated with Garmin GPS map76CSx, and averages of minimum and maximum temperature for each population were extracted from web site of meteorology organization of Iran.

The mean of morphological characters were determined for populations. For grouping of populations, data were standardizes (mean = 0, variance = 1) and used for multivariate analyses including UPGMA (Unweighted Paired Group using Average method), Average Linkage (within group) and Principal Coordinate Analysis (PCO)[12]. One-way ANOVA test performed for assessing significant difference for quantitative characters between populations. Pearson correlation coefficients of was determine among quantitative morphological characters with ecological features of the populations habitat including longitude, latitude, elevation and average of minimum and maximum temperature per year to show their possible relationship between them. MVSP ver.3.1 (2004) and SPSS ver. 9 (1998) softwares were used for statistical analyses.

Results and Discussion

Intrapopulation study: For intrapopulation investigations, twenty two quantitative and qualitative morphological characters of three to four individuals of each population were studied (table-2). Qualitative morphological features varied between individuals and ANOVA test performed between these characters showed significant difference ($P<0.05$) for some features such as pedicle length, basal leaves length/ width ratio, floral leaves length, calyx length/ width ratio, calyx dent width and the number of flower cycle in inflorescent (table-3), but one-sample T-test showed significant difference for all examined characters (table-4). Furthermore quantitative characters varied between individuals of some populations. For example basal leaves shapes were in the shapes of linear, lanceolate, oblanceolate or ovate and this character varied between individuals of all populations with the exception of Ghargh Abad and Vardeh populations. In contrast, floral leaves shapes were nearly stable between individuals of populations and had minor variations and were found in the shape of lanceolate and rarely linear or ovate.

Significant correlations positive or negative were occurred between morphological features of individuals with ecological factors of habitat. For example, a significant positive correlation ($r=0.29$, $p<0.05$) found between stem height with eastern distributions of plants. Pedicle length had significant positive correlations ($p<0.01$) with northern and eastern distribution and also with elevation of habitat ($r=0.33$, $p<0.05$), but this character had a significant negative correlation ($r= - 0.39$, $p<0.01$) with maximum temperature of day. A significant negative correlation ($r= - 0.26$, $p<0.05$) occurred between floral leaves length with minimum temperature of day, and a significant positive correlation ($r= 0.48$, $p<0.01$) found between this features with habitat elevation. Significant positive correlations ($p<0.01$) occurred between calyx dimensions (length and width) with eastern distributions, but significant negative correlations ($p<0.05$) were found between these features with elevation. Corolla length has significant negative correlations ($r= - 0.31$, $p<0.05$) with eastern distribution as well maximum temperature of day.

Studied individuals were separated from each others in Average Linkage (within group) tree of morphological features (figure-1). PCO plat as well as PCA plot showed high difference between individuals of populations, especially in Haraz and Nimvar populations (figure- 2, 3). Individuals of all populations were far from others in the mention diagrams, this subject confirmed high variations in individual's morphological characters.

Interpopulation study: In this section average amounts of each morphological character were used for examination of variations between populations (table-3). Totally twenty two qualitative and quantitative morphological characters were investigated. Some populations had distinct characters which distinguished from others, for example, Haraz population had largest stem and pedicle with longest and widest floral leaves. Shortest basal leaves, smallest basal and floral leaves length/ width ratio, widest calyx and also biggest calyx dent were found in Ivanaki population. This condition was true about Joshaghan population which shortest calyx occurred in it.

Significant correlations, positive or negative, were present between average amounts of morphological characters with ecological factors of habitat. For example, a significant negative correlation (r= - 0.64, p<0.01) found between calyx dent lengths with habitat elevation, but this character had a significant positive correlation (r= 0.68, p<0.01) with maximum temperature of day. Calyx width had a significant positive correlation (r= 0.79, p<0.01) with eastern distribution of populations. Significant positive correlations found between floral leaves length (r= 0.56, p<0.05) and width (r= 0.72, p<0.01) with eastern distribution of populations.

Studied populations were different in morphological characters and separated from each other in PCA, CA and PCO plots as well as UPGMA tree (figure- 4 to 7).

Table-2
ANOVA test of some quantitative morphological characters

		Sum of Squares	df	Mean Square	F	Sig.
Stem height	Between Groups	236.650	4	59.162	1.639	.178
	Within Groups	1948.732	54	36.088		
	Total	2185.381	58			
Pedicle length	Between Groups	.316	4	.079	7.084	.000
	Within Groups	.601	54	.011		
	Total	.917	58			
Basal leaf length/ width ratio	Between Groups	11.195	4	2.799	4.959	.002
	Within Groups	30.477	54	.564		
	Total	41.672	58			
floral leaf length	Between Groups	1.282	4	.321	2.544	.050
	Within Groups	6.805	54	.126		
	Total	8.087	58			
calyx length/ width ratio	Between Groups	1.402	4	.350	3.654	.010
	Within Groups	5.178	54	.096		
	Total	6.579	58			
Corolla width	Between Groups	.310	4	.077	1.656	.174
	Within Groups	2.526	54	.047		
	Total	2.836	58			
Calyx dent. length	Between Groups	.025	4	.006	2.363	.065
	Within Groups	.142	54	.003		
	Total	.167	58			
Calyx dent. width	Between Groups	.028	4	.007	2.524	.051
	Within Groups	.149	54	.003		
	Total	.177	58			
Flower no.	Between Groups	5.313	4	1.328	2.325	.068
	Within Groups	30.856	54	.571		
	Total	36.169	58			
Cycle no.	Between Groups	26.157	4	6.539	4.929	.002
	Within Groups	71.639	54	1.327		
	Total	97.797	58			

Table-3
One-Sample T-Test of quantitative morphological features

					95% confidence interval of the difference	
Characters	**T**	**D.F**	**Sig. (2-tailed)**	**mean difference**	**lower**	**upper**
Stem height	37.943	58	.000	30.3220	28.722	31.922
Pedicle length	10.717	58	.000	.17542	.1427	.2082
Basal leaf length	24.747	58	.000	2.0635593	1.896643	2.230475
Basal leaf width	32.112	58	.000	.7118644	.667490	.756239
Basal leaf length/ width	26.966	58	.000	2.97576	2.7549	3.1967
Floral leaf length.	25.173	58	.000	1.2237288	1.126420	1.321037
floral leaf width	29.611	58	.000	.48559	.4528	.5184
floral leaf length/ width	36.653	58	.000	2.5530508	2.413622	2.692480
calyx length	58.742	58	.000	1.2491525	1.206586	1.291719
calyx width	42.094	58	.000	.5686441	.541603	.595685
calyx length/ width	51.004	58	.000	2.2364407	2.148669	2.324212
Corolla length	59.587	58	.000	1.4054237	1.358211	1.452636
Corolla width	24.541	58	.000	.7064407	.648820	.764062
Corolla length/ width	23.431	58	.000	2.1391525	1.956400	2.321905
Calyx dent length	29.930	58	.000	.20932	.1953	.2233
Calyx dent width	24.283	58	.000	.17458	.1602	.1890
Calyx dent length/ width	24.256	58	.000	1.27593	1.1706	1.3812
Flower no.	57.207	58	.000	5.88136	5.6756	6.0872
Cycle no.	27.371	58	.000	4.62712	4.2887	4.9655
cyc.dis	29.699	58	.000	3.2203390	3.003285	3.437393

Table-4
Some of important morphological characters of studied populations (all values are in cm)

Populations	**Ste.hi**	**ba.le.sh**	**pedi**	**ba.le.le**	**ba.le.wi**	**fl.le.sh.**	**fl.le.le.**	**fl.le.wi**	**cal.wi**	**cor.le.**	**cor.wi**	**den.wi**	**flow.no**	**cyc.no**	**cyc.dis**
J. Boein	29	Lanceolate	0.2	2.87	0.9	Lanceolate	1.02	0.5	0.6	1.35	0.47	0.17	7.25	4.8	2.67
Jasb	33.87	Ovate	0.2	2.67	0.75	Lanceolate	1.52	0.6	0.6	1.4	0.9	0.22	5.75	6.5	3.57
Rangraz	25.87	Lanceolate	0.12	2.4	0.55	Lanceolate	1.22	0.4	0.6	1.27	0.6	0.18	6	4.8	2.52
Abas Abad	31.16	Oblanceolate/ ovate	0.2	2.36	0.83	Lanceolate	1.36	0.5	0.5	1.3	0.6	0.16	5.66	5.3	3.83
Vidar	27.87	Lanceolate	0.12	2.32	0.75	Lanceolate	1.22	0.4	0.5	1.4	0.77	0.15	5	4	2.82
Haraz	36.62	Linear-oblong	0.42	2.22	0.72	Lanceolate	2.1	0.7	0.7	1.37	0.67	0.15	6	3.8	3.17
Peyghambar	33.5	Oblanceolate	0.2	2.02	0.85	Lanceolate	1.07	0.5	0.6	1.47	0.8	0.15	5.5	5.3	3.12
Vardeh	29	Lanceolate	0.07	2	0.67	Lanceolate	1.2	0.6	0.5	1.4	0.72	0.2	5.5	4.5	3.35
Nobaran	28.75	Linear	0.2	1.85	0.72	Lanceolate	1.2	0.5	0.5	1.42	0.7	0.17	6	5.3	3.37
Salafchegan	29	Oblanceolate	0.15	1.85	0.62	Lanceolate	1.05	0.4	0.6	1.72	0.7	0.2	5.75	3.8	2.72
Joshaghan	27.25	Lanceolate/ Ovate	0.17	1.82	0.77	Lanceolate	1.2	0.4	0.5	1.45	0.52	0.15	5.75	4.3	3.52
Abegarm	27.12	Oblong	0.2	1.72	0.7	Lanceolate	1.17	0.4	0.5	1.25	0.7	0.2	6	4.5	3.2
Nimvar	34.75	Ovate	0.02	1.67	0.55	Linear	1.15	0.6	0.6	1.52	0.9	0.2	5.75	4.3	4.55
Ghargh Abad	29.5	Linear	0.1	1.65	0.52	Lanceolate	0.77	0.3	0.5	1.32	0.63	0.1	6	4.3	2.62
Ivanaki	31.75	Linear	0.23	1.45	0.77	Lanceolate	1.1	0.6	0.8	1.35	0.85	0.23	6.25	4.5	3.37

Abbreviations: ste.hi: stem height, ba.le.sh: basal leaves shape, pedi: pedicle length, ba.le.le: basal leaves length, ba.le.wi: basal leaves width, fl.le.sh: floral leaves shape, fl.le.le: floral leaves length, fl.le.wi: floral leaves width, cal.wi: calyx width, cor.le: corolla length, cor.wi: corolla width, den.wi: calyx dent width, flow. no: flower no. in cycle, cyc.no: number of floral cycle, cyc.dis: distance between flower dicks.

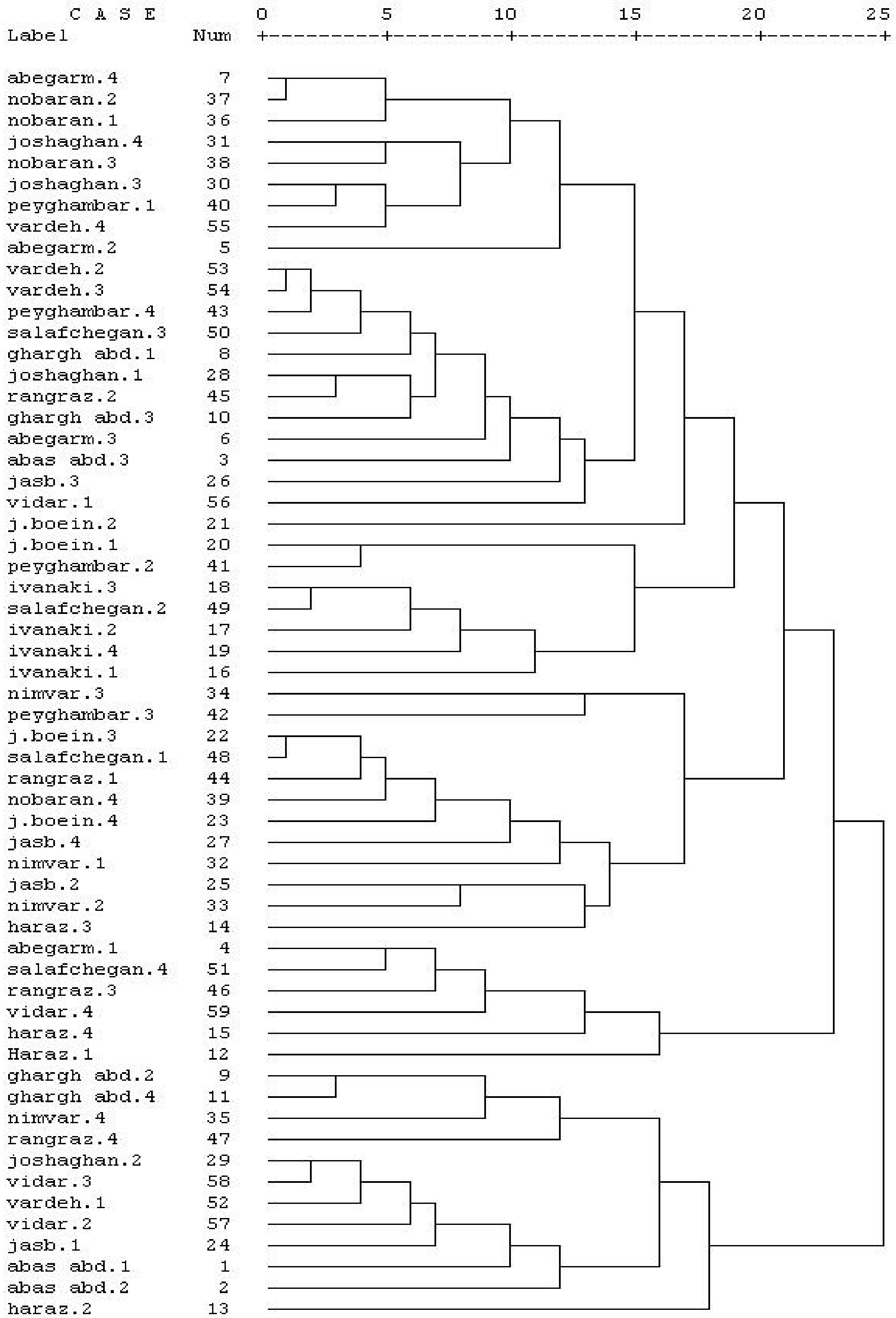

Figure-1
Morphological Average Linkage tree(within group) of studied individuals of populations

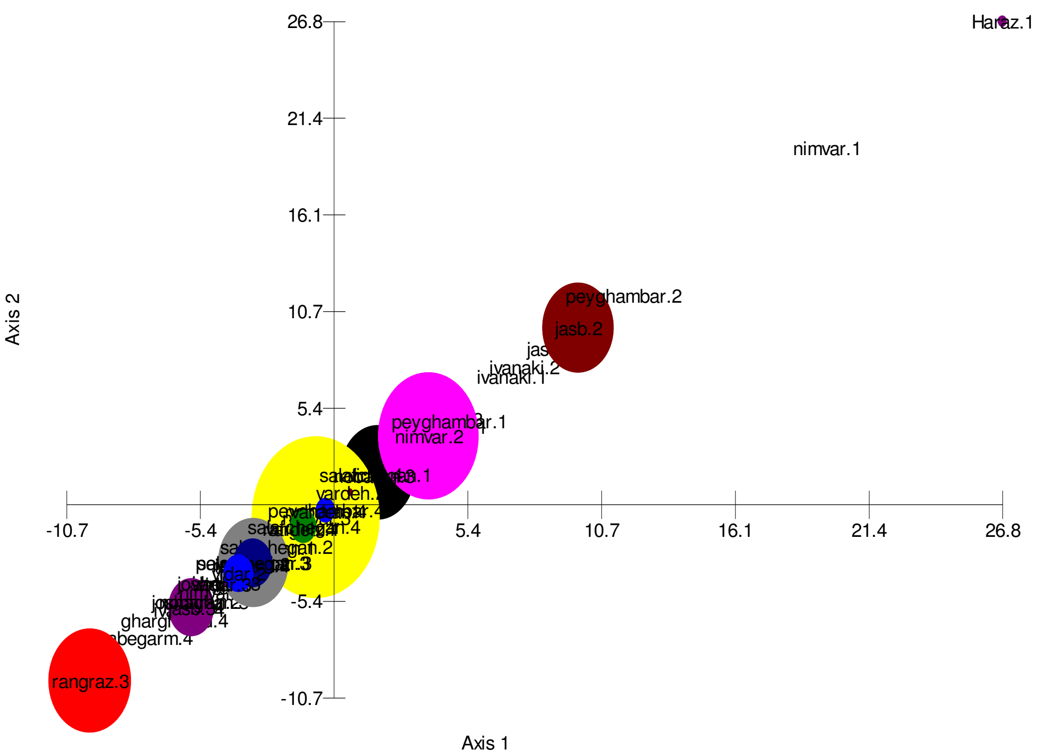

Figure-2
PCO plot of indiviuals of studied populations based on morphological characters

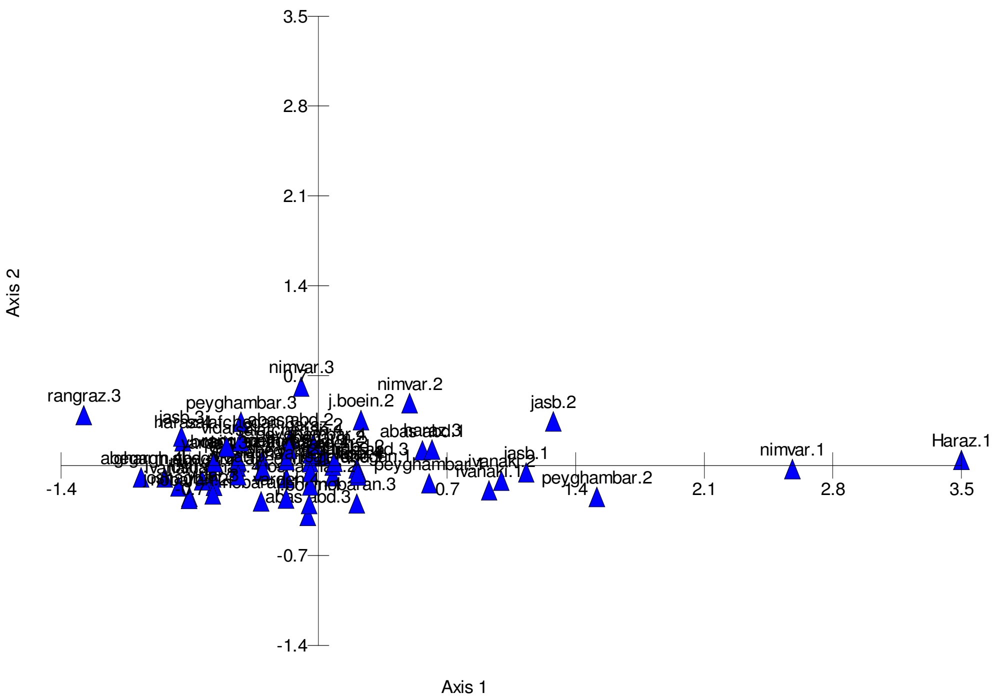

Figure-3
PCA plot of indiviuals of studied populations based on morphological characters.

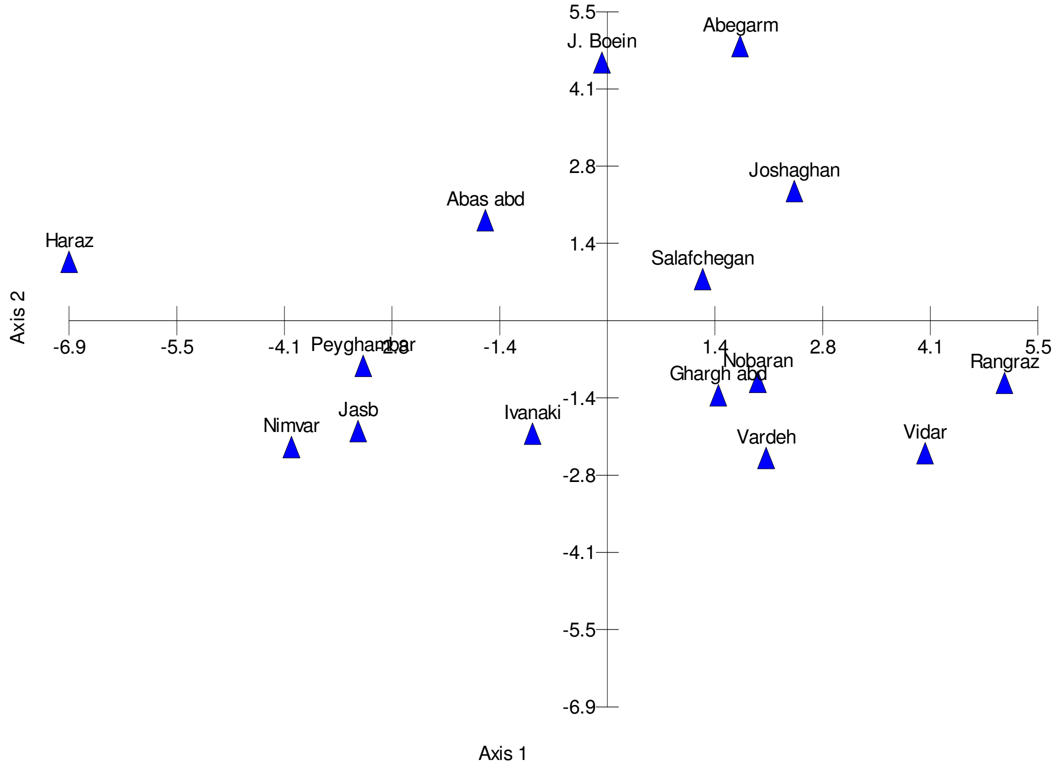

Figure-4
PCA plot of studied populations based on morphological characters

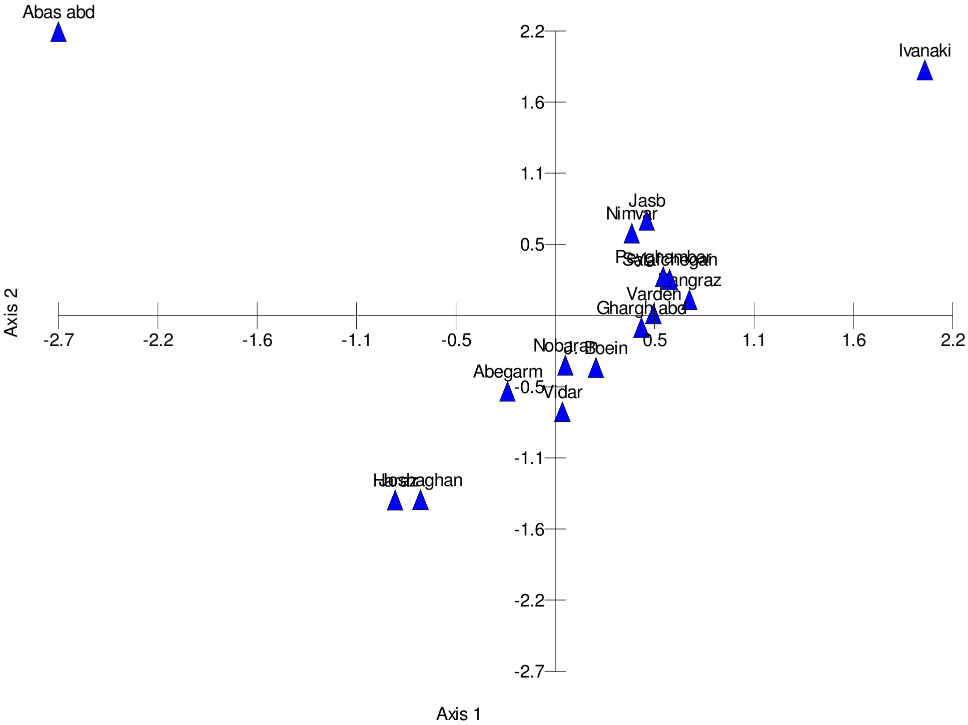

Figure-5
CA plot of studied populations based on morphological characters

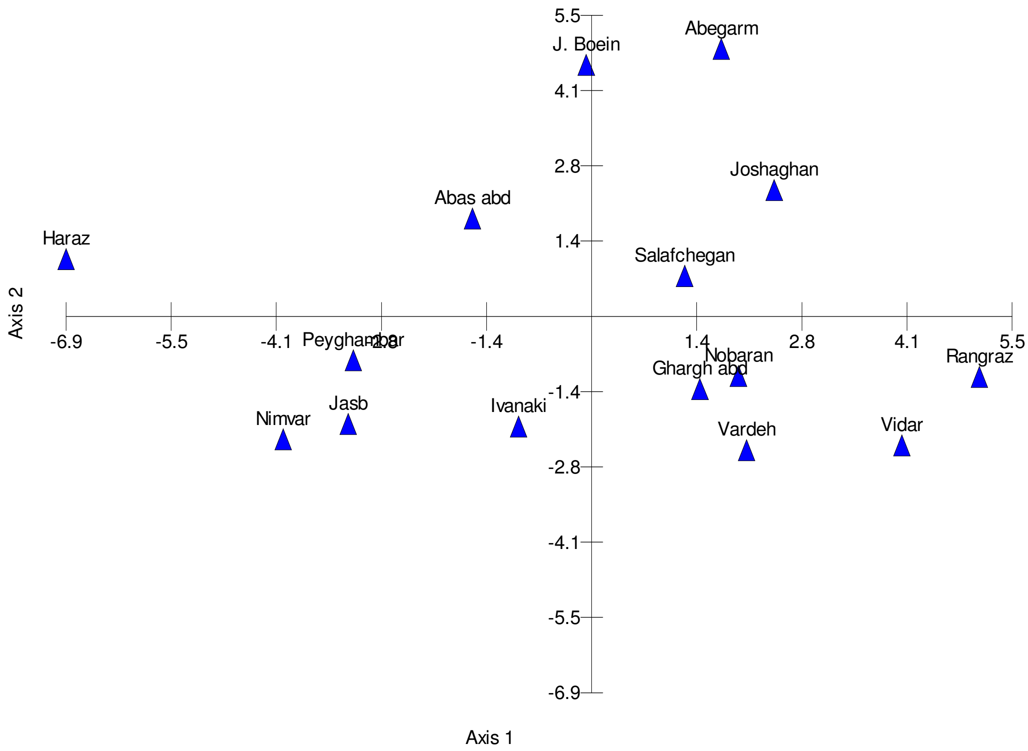

Figure-6
PCO plot of studied populations based on morphological characters

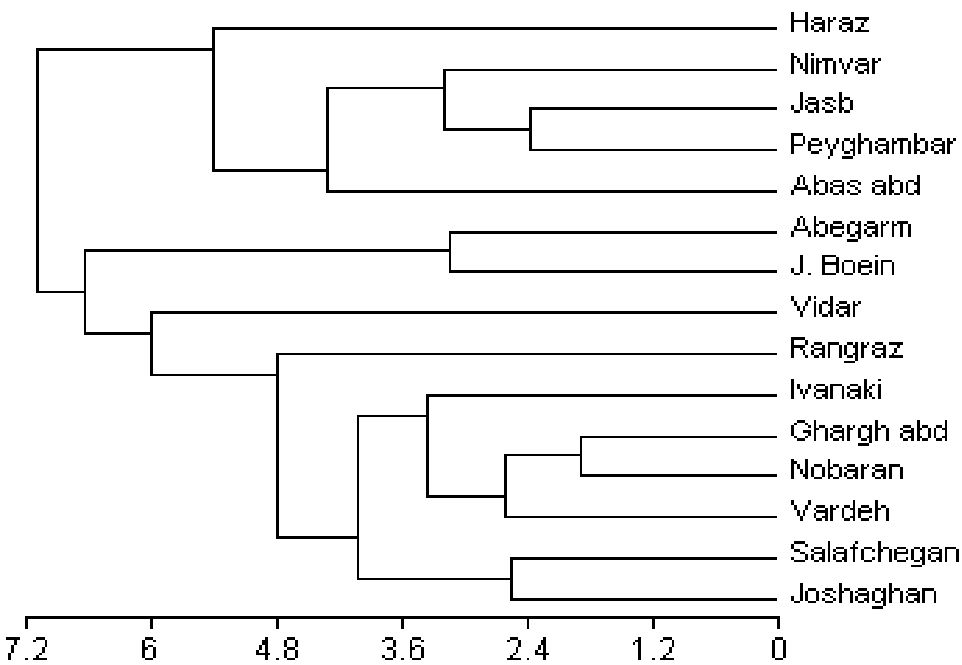

Figure-7
Morphological UPGMA tree of studied populations

Ecological study: Five environmental factors were examined for each habitat including: maximum and minimum temperature for days, elevation of habitats and their longitude and altitude. All of the studied population's habitats were belong to Irano-Touranian region. This region is very widespread in Iran and has many subprovinces. Among studied populations thirteen stations occurred in Kurdo-Zagrosian subprovince, but Haraz and Ivanaki populations growth in different subprovinces. Ivanaki population belongs to ecoton area between Atropatenian and central Iranian subprovinces. Haraz population found in ecoton area between Irano-Touranian region with Euxino-Hyrcanian province of Euro-Siberian region. Ecological factors differed between stations; therefore habitats were separated in ecological CA plot (figure-8) and also UPGMA tree (figure-9). In the mentioned diagrams Haraz, Ivanaki and also Abegarm habitats were far from others. This condition confirmed difference and also variations between habitats.

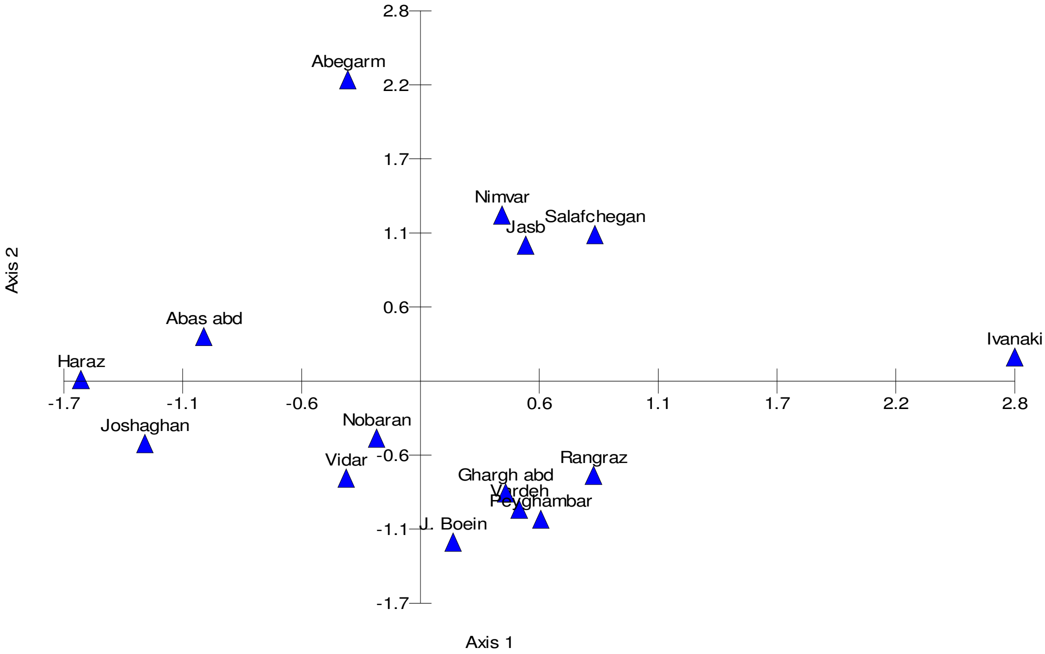

Figure-8
CA. plots of habitat ecological factors

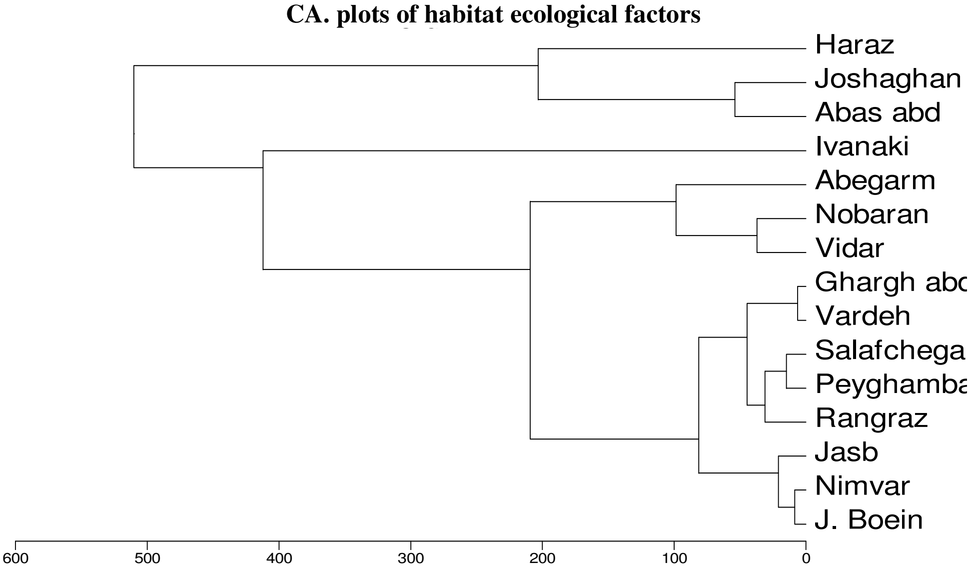

Figure-9
UPGMA tree of habitats ecological parameters

Discussion: Plasticity in phenotypic characters is naturally abundant, and often contains morphological, physiological as well life-historical characters of organism. As a result, this phenomenon changes numerous interactions between organism with their biotic and abiotic factors of environments. In present study the phenotypic plasticity of *Stachys inflata* was investigated. For this purpose morphological traits were examined between (inter) and within (intera/individuals) populations.

Quantitative morphological characters varied inter and intrapopulation. The ANOVA test and also T-test confirmed significant variations. This phenomenon confirmed the effect of different environmental characters on plant phenotype. For example, significant differences were found in floral leaves length and also basal leaves length/ width ratio, which was importance for determination of leaves shape. Beside to proportionate allocation and dynamic developmental responses, plants show plasticity for some of morphological characters, such as structure and size of organ. One ecologically important example is the leaves sizes generated in different light conditions. Supposed that reduced light availability inevitably decreases the total number of produced leaves in plants, another adaptive response to low light is to shape leaves of individual as large as possible under those growth limits to maximize surface area for light capture. For example, the species of *Polygonum* differed in this aspect of plasticity, since in *P. hydropiper* individuals grown in shade maladaptively 40% of leaf size decreased in comparison with plants growth in full sun, whereas light-deprived individuals of the widely distributed *P. persicaria* maintained close to equivalent leaf size. In fact, decrease in leaf specific area under shade condition is one of the most well-known and universal aspects of morphogenetic plasticity in plants[13, 14]. For example, individual genotypes from two field populations of *P. persicaria* increased specific leaf area nearly 2- fold at moderate light conditions and 2.5- to 3-fold at very low light habitat, in comparison to the far thicker leaves generated at full sun[15].

In addition to vegetative organs, reproductive organs such as flowers characters may be varied between populations. These kinds of variations are essential for preservation of reproductive ability via different habitat conditions which populations occurred on it. In the studied populations significant variations were found in some reproductive organs such as calyx length/ width ratio, calyx dent width and the number of floral cycle in inflorescent. Variation in the aspects of plasticity influence the abilities of different taxa to preserve reproduction in resource-poor stressful conditions and/or to maximize reproductive output in favorable conditions, both of these are important factors in ecological breadth[16-18].

Variations were occurred in morphological traits of population's individuals. Samples of populations were separated from others and placed separately. Highest variations were present in Haraz and Nimvar populations. for the reason that plasticity can change a variety of direct and indirect interactions among individuals and their habitat , it should ultimately affect many ecological processes, for example population and community dynamics, and aspects of community and ecosystem functioning. Many studies, in a wide range of taxa, have shown that plasticity impresses direct interactions between individuals and biotic (e.g. predation, herbivory and competition) and abiotic (e.g. temperature and light) elements of their environments in a variety of ways[8,9,19-21].

The mentioned intrapopulation variations may be related to ecological conditions of different niches which present in habitats. The regional terms of niche can be very different from general ecological conditions of habitat; therefore these factors affected phenotypical features of plant that occurred under these conditions. Many organisms have the ability to convert the environments that they test, called 'niche construction[22]. Recently, experiment has confirmed that plasticity can result in niche construction and it is especially interesting when environmental alternations lead to improvements[23]. For example, many plants elongate shoot internodes to overtop shading that created by neighboring plants, this developmental response brings the photosynthetic organs of that individual into a more favorable light environment. In *Arabidopsis thaliana*, shading by neighboring plants also products a variation in phenology by accelerating reproductive onset. This plastic response enhances the resource environment of the plant during the crucial period of seed and fruit maturation, because, in most environments, light and nutrient availability decline later in the growth season[24]. The investigation on phenotypic plasticity influencing niche construction has originally focused on plants and the effects of morphological and life-history plasticity[23,24]. This examination shows that individual–environment interactions might be more complex than was previously thought.

The interpopulation variations may be related to habitat of populations. In some case, the arrangements of populations in morphological and ecological plots and trees were similar. For example, in UPGMA trees of morphological character and also ecological factor Ivanaki and Haraz populations were separated from others, it may be related to phytogeographical features of these populations. The kind of subprovince of Ivanaki and Haraz populations differed from others. Ivanaki and population belongs to ecoton area of Atropatenian and central Iranian subprovinces, while Haraz population found in ecoton area between Irano-Touranian region with Euxino-Hyrcanian province, but others were belong to Kurdo-Zagrosian subprovince. The arrangements of some populations were alike in CA plot of morphological characters and ecological factors, for example, Jasb, Nimvar, and Abas Abad.

The plasticity in morphological characters enables individuals of populations to establish in different habitat, and these types of variations provide material for evolution of new taxa. One of the potentially important mechanisms for facilitating macroevolution is named phenotype plasticity[8,25]. This mechanism can happen through at least two pathways: when a population occupies a new habitat, plasticity can result in the genetic assimilation of a feature. Essentially, pre-existing variation for plasticity could enable a population to persist under new conditions, even though the population might be sub-adapted to them. Necessarily, pre-existing difference for plasticity could enable a population to insist under new condition of habitat, even though the population might be sub-adapted to these ondition. Such resistant would then give time for new genetic variation to product via mutations or recombination, and for natural selection to accelerate the adaption to the new conditions. If the new conditions continue, selection might favor a deduction in plasticity, essentially genetically assimilating the character(s).

Conclusion

Each species, on the basis of its distribution, consist of one till many populations and each population has individuals which distribute in its habitat. *Stachys inflata* is a widespread species that occurs naturally in various habitats. The results of this study showed that different populations of same species that grow under different ecological conditions, altered their morphological features for adaption with their habitat condition. Different populations of same which occur in habitat with similar ecological characters have similarity in morphological traits. The process in which plant morphological characters varied in different ecological condition, in order to adaption with their habitats, is named phenotypic plasticity. Not only morphological features of plant samples varied between populations, but also this kind of differentiation presented within a population or on the other hand between members of a population. It might be possible related to ecological niche. Habitat of a population may be consisting of different microhabitats and different ecological condition prevailing on them. These conditions can yield different morphological characters within a population and lead to interpopulation variation or polymorphism.

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