Acta Biologica Sibirica
Vol 5 No 1 (2019): Acta Biologica Sibirica, 167-174
Articles

Species diversity of tree plantations in industrial enterprise protective zones (Zaporizhzhya, Ukraine)

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Some 49 species of woody plants were identified among the tree plantations in sanitary protection zones of industrial enterprises in Zaporizhzhya (southeastern Ukraine); 10 species of tree plants are present in all the sanitary protection zones. The number of plant species in protective plantings varies considerably from 11 ("Zaporizhskloflus") to 30 species ("Zaporizhstal"). The similarity of the species composition in plantations was assessed using the Jaccard and Sørensen indices. We calculated the species richness by Margaleff and Shannon indices, the dominance indices were calculated according to Simpson and Berger-Parker. The vast majority of forest belts in the sanitary protection zones of Zaporizhzhya industrial enterprises require the species enrichment, taking into account the tolerance of species to various pollutants.

Introduction

In nature, the creation of infra−specific difference can occur through a wide range of mechanisms such as local adaptation, phenotypic plasticity, parental conditions and artificial selection [22]. West-Eberhard [23] has believed that when it is resulted from plasticity, features can differ rapidly within generations and vary drastically across populations in dissimilar habitats. Recently, studies have broadened in the identification of variation to incorporate the considerable phenotypic difference within and among populations of the same species [22].

Investigations have showed that difference of phenotypic feature within species can be as extreme as the characteristic difference across species [1]. Moreover, infra−specific difference may influence structure of community and function of ecosystem as much as difference among species [6,14].

Salvia limbata is an aromatic herb of Lamiaceae that grows naturally in various regions of Iran and some neighboring countries [7,16]. Different flavone compounds such as ladanein, salvigenin, luteolin 7−methyl ether, cirsiliol, eupatorin, luteolin 7−O−glucoside and rosmarinic acid were isolated from extracts of this plant [5]. Moreover, Saeidnia et al. [17] have suggested that *S. limbata* can accumulate tryptophan, sterols and their glucosides, therefore, consumption of this plant as an herbal tea or other preparations might be useful for dietary deficiency of this amino acid. In mice, *S. limbata* has beneficial effects to decrease dependence sign produced by morphine and increase in pain threshold compared with the control [2].

Although, comparative infraspecific anatomical [12] and phytochemical studies [10,13] have been performed in this species, as far we could search, no infra−specific morphological investigation is available for its populations.

In current study, we studied morphological characteristics of inflorescence in eight populations of *S. limbata*, in order to evaluate pattern of morphological variations among the populations and also find possible phenotype(s) among the studied populations.

Material and methods

Morphological studies

In current study, eight natural populations of *S. limbata* were harvested from different regions of the country (Table 1), and were identified according to descriptions provided in Flora of Iran [8]. Morphological differences were carefully evaluated for reproductive organs from collected samples of the studied populations. The morphological characteristics of flowers were studied under dissecting stereo microscopes. For morphometric analysis, 80 plant specimens from 8 populations were used. Voucher specimens were deposited at Herbarium of Arak University.

coding	Locality address
1	Markazi province, arak, Sardasht, 1850 m.
2	Markazi province, Saveh, Sangak, 1940 m.
3	Khorasan Razavi, Semnan to Ghochan, 1730 m.
4	Markazi province, Zarandiyeh, Vidar, 1750 m.
5	Semnan province, Ahovan, 1720 m.
6	Markazi province, Zarandiyeh, 1430 m.
7	Semnan province, Ghochan to Semnan
8	Tehran province, Gajroud, 1600 m.

Table 1. *Locality address of the studied populations of S. limbata*

Character measurements

Measurements of morphological traits were performed on each plant, including its flowering stem with flowers in bloom. In total, 21 qualitative features were measured on each specimen. Each character was measured four times per each plant sample, and their average determined. The studied characteristics were: calyx width, length and calyx length / width ratio, petal length, width and petal length / wide ratio, calyx short teeth length, calyx short teeth width, calyx short teeth length / width ratio, style length, stigma length, calyx long teeth length, calyx long teeth width, calyx long teeth length / width ratio, anther length, anther width, anther length /width ratio, pedicle length, long filament length, short filament length, long /short filament length ratio.

Statistical analyses

Morphometric data was subjected to one–way analysis of variance (ANOVA) to determine if significant difference existed among populations for each feature measured. Mean and standard deviations of characteristics were tacalculated. These analyses were performed using SPSS ver. 17. Cluster analysis was carried out based on quantitative features using UPGMA, PCO, PCA and C.A–Joined plots clustering in Multivariate Statistical Package (MVSP) program [15].

Results

The mean and standard deviation of the studied morphological variables were presented in Table 2 .

Population	Anther length	Antler width		Stigma length	Style length	Short Filament length	Long filament length	Petal length	Petal width	calyx Short teeth length	calyx Short teeth width	calyx long teeth length	calyx long teeth width	Calyx length	Calyx width
1	Mean	4.00	2.00	5.50	2.90	19.60	2.70	3.90	9.80	5.00	2.00	0.14	2.60	0.30	9.90

	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	SD	0.47	0.00	1.17	0.31	5.12	0.48	0.56	4.34	0.66	0.00	0.05	0.51	0.00	1.79
2	Mean	5.10	2.00	1.90	2.65	19.70	2.80	3.55	12.50	5.10	1.15	0.94	2.50	1.25	9.70
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	SD	0.31	0.00	0.73	0.24	2.71	0.34	0.49	2.32	0.56	0.47	0.63	0.74	0.54	0.82
3	Mean	4.80	2.00	5.60	2.45	17.40	2.45	3.35	11.60	4.50	0.66	0.36	1.90	0.82	9.00
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	SD	0.34	0.00	0.84	0.28	1.34	0.43	0.47	1.83	0.52	0.41	0.28	0.61	0.47	0.66
4	Mean	4.55	2.00	4.05	1.90	20.60	2.80	3.60	12.50	4.80	0.50	0.43	2.35	0.64	8.90
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	SD	0.59	0.00	0.95	0.61	6.23	0.25	0.51	3.53	0.42	0.35	0.24	1.00	0.20	0.87
5	Mean	5.20	2.10	2.20	2.05	14.60	2.00	2.75	8.10	3.70	0.64	0.33	2.75	0.20	9.00
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	SD	0.42	0.31	0.91	0.76	0.51	0.66	0.35	1.44	0.48	0.30	0.21	0.67	0.14	0.66
6	Mean	4.70	2.00	4.50	1.95	18.50	2.90	3.85	11.60	5.00	0.68	0.28	2.45	0.36	8.70
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10

	SD	0.48	0.00	0.84	0.59	3.86	0.21	0.33	1.26	0.47	0.34	0.16	0.72	0.24	0.48
7	Mean	4.90	2.10	4.80	2.60	17.20	2.70	3.38	10.90	5.20	0.23	0.27	1.01	0.38	7.80
	N	10	10	10	10	10	10	9	10	10	10	10	10	10	10
	S.	0.21	0.31	1.03	0.45	2.14	0.34	0.48	1.44	0.42	0.12	0.18	0.44	0.19	1.61
8	Mean	4.75	2.20	2.80	2.30	17.10	2.40	3.20	10.40	4.90	0.45	0.42	2.95	0.92	9.20
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	SD	0.42	0.42	0.63	0.53	2.99	0.39	0.42	2.41	0.87	0.47	0.34	1.14	0.69	1.39

Table 2. *Mean and standard deviations of some morphological characteristics of studied populations (all values are in mm).*

Morphological traits differed among the studied populations. Biggest and smallest anther lengths were recorded in population no. 5 and 1, respectively. While, reverse pattern were recorded for long filament length. Therefore, largest and shortest long filament lengths were reported for populations no. 1 and 5, respectively. Maximum and minimum lengths of pedicle were belonged to population's no. 3 and 2, respectively. Largest stigma was observed in population no. 1, but population no. 4 had smallest stigma. Longest style was found in population no. 4, and shortest in population no. 5. Maximum and minimum petal lengths were registered in populations no. 5 and 2, respectively. Population no. 1 had longest calyx and widest calyx short teeth; however, shortest calyx and narrowest calyx short teeth were belonged to population no. 7. Largest and smallest short filament length was found in populations no. 6 and 5, respectively (Fig. 1).

Fig. ***Fig. 1.*** *Image of Salvia limbata and its reproductive organs. A) Flowering stem, B) inflorescence cycle, C) lateral view of flower, D) calyx, E) fertile and sterile anther, F) stigma, style and ovary.*

Furthermore, the ANOVA test confirmed significant variations ($p \leq 0.01$) for all studied variables, except for anther width (Table 3).

Characteristics		Sum of Squares	df	Mean Square	F	Sig.
Anther length	Between Groups	9.573	9	1.064	5.741	0.000
	Within Groups	16.675	90	0.185		
	Total	26.248	99			
Anther width	Between Groups	1.010	9	0.112	1.836	0.072
	Within Groups	5.500	90	0.061		
	Total	6.510	99			
Anther	Between Groups	2.387	9	0.265	3.696	0.001

length/width ratio						
	Within Groups	6.457	90	0.072		
	Total	8.844	99			
Pedicle length	Between Groups	171.202	9	19.022	22.698	0.000
	Within Groups	75.425	90	0.838		
	Total	246.628	99			
Stigma length	Between Groups	10.962	9	1.218	5.420	0.000
	Within Groups	20.225	90	0.225		
	Total	31.187	99			
Style length	Between Groups	277.690	9	30.854	2.654	0.009
	Within Groups	1046.500	90	11.628		
	Total	1324.190	99			
Short Filament length	Between Groups	6.772	9	0.752	4.463	0.000
	Within Groups	15.175	90	0.169		
	Total	21.947	99			
Long filament length	Between Groups	11.089	9	1.232	6.199	0.000
	Within Groups	17.689	89	0.199		
	Total	28.778	98			
Petal length	Between Groups	171.200	9	19.022	3.524	0.001
	Within Groups	485.800	90	5.398		
	Total	657.000	99			
Petal width	Between Groups	18.840	9	2.093	6.587	0.000
	Within Groups	28.600	90	0.318		
	Total	47.440	99			
Petal length/width ratio	Between Groups	4.134	9	0.459	2.408	0.017
	Within Groups	16.975	89	0.191		
	Total	21.109	98			
Calyx Short teeth length	Between Groups	22.404	9	2.489	13.065	0.000
	Within Groups	17.148	90	0.191		
	Total	39.552	99			
Calyx Short teeth width	Between Groups	4.532	9	0.504	6.069	0.000
	Within Groups	7.468	90	0.083		
	Total	12.000	99			
Calyx Short teeth length/width ratio	Between Groups	2290.906	9	254.545	11.111	0.000
	Within Groups	2038.907	89	22.909		
	Total	4329.813	98			
Calyx long teeth length	Between Groups	41.789	9	4.643	8.759	0.000
	Within Groups	47.709	90	0.530		
	Total	89.498	99			
Calyx long teeth width	Between Groups	11.206	9	1.245	9.699	0.000
	Within Groups	11.554	90	0.128		
	Total	22.760	99			
Calyx long teeth length/width	Between Groups	31225.271	9	3469.475	11.935	0.000

ratio						
	Within Groups	26163.531	90	290.706		
	Total	57388.802	99			
Calyx length	Between Groups	84.810	9	9.423	6.812	0.000
	Within Groups	124.500	90	1.383		
	Total	209.310	99			
Calyx width	Between Groups	106.462	9	11.829	10.622	0.000
	Within Groups	100.225	90	1.114		
	Total	206.688	99			
calyx length/width ratio	Between Groups	7.300	9	0.811	10.565	0.000
	Within Groups	6.910	90	0.077		
	Total	14.211	99			

Table 3. *Results of ANOVA test of quantitative morphological features among the populations*

The studied populations were clustered separately in UPGMA tree (Fig. 2); moreover, PCA and PCO (Fig. 3,4) plots produced similar outputs.

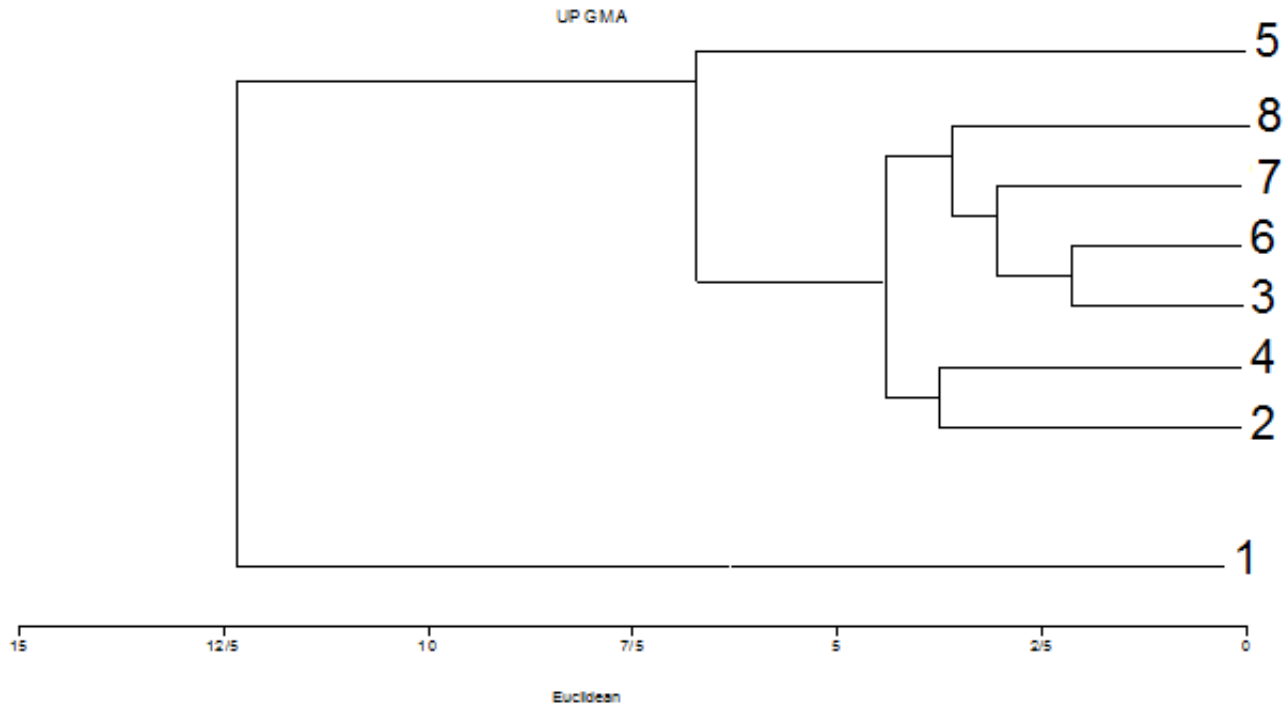

Fig. 2. *UPGMA tree of the studied populations based on morphological variables.*

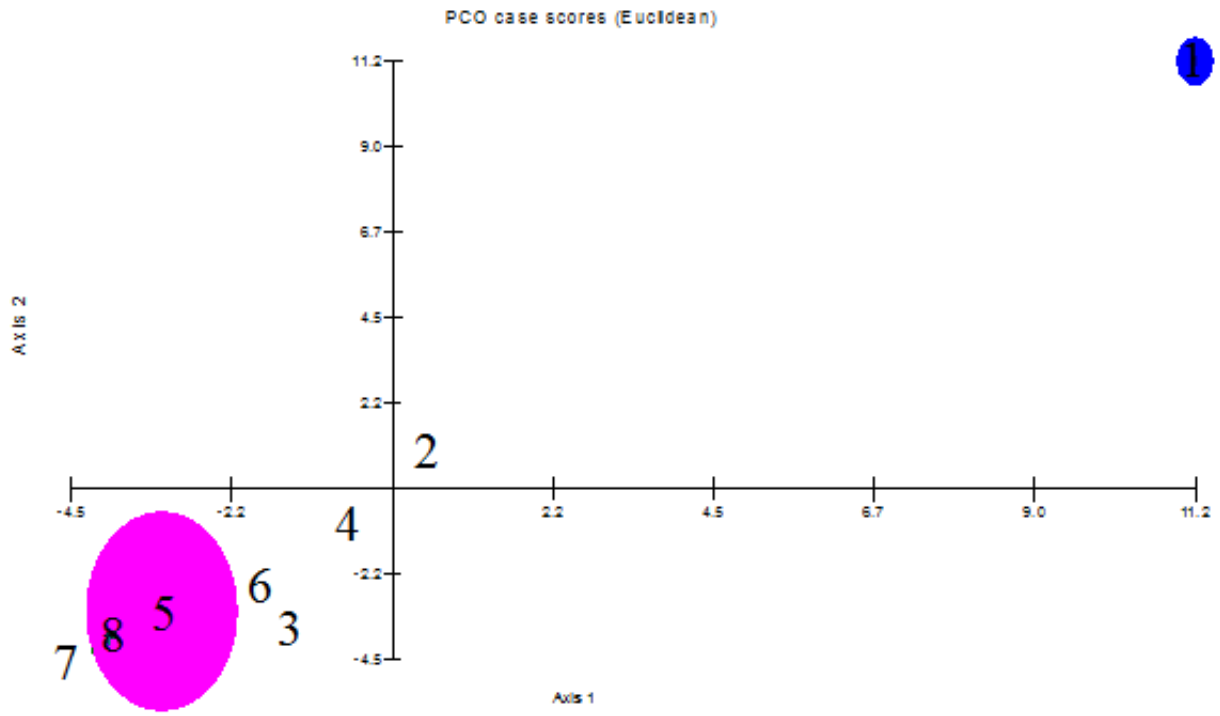

Fig. 3. *PCO plot of the studied populations of S. limbata based on morphological features.*

Therefore, population's arrangement in the tree was discussed here. *The tree had two branches: we found population no. 1 in small branch, while other populations were observed in large branch, which was divided into two sub–branches. Population no. 5 placed far from others in a sub–branch and the rest populations were registered in another sub–branch into two groups. Populations no. 2 and 4 were observed as a pair. Eventually, in the other sub–group populations no. 3 and 6 were close together and populations no. 7 and 8 gradually joined them. Therefore, we had four distinct morphological groups among the studied populations.*

Fig. 4. PCA plot of the studied populations based on morphological characteristics.

CA. joined plot (Fig. 5) revealed each of these groups were characterized by special features that were useful in identification of them. For example, calyx length, calyx short teeth length and calyx long teeth length were prominent variables for populations no.1. Moreover, populations no. 2 and 4 were characterized by style length, calyx short teeth length/width ratio and calyx long teeth length/width ratio. Populations no. 5 was identified according to calyx length/width ratio**.**

Discussion

In this research, we evaluated morphological variables of inflorescence in the selected populations of *S. limbata*. Because, these characteristics are more stable than vegetative characteristics [19], in addition, Jamzad [8] used inflorescence characteristics along with palynological as well as ITS data in her valuable work on Iranian Lamiaceae species, Flora of Iran.

Morphological features highly varied among the studied populations. Furthermore, previous studies have showed phytochemical and anatomical variations among various populations of *S. limbata*. For example, Mirza et al. [13] identified germacrene D (25.7%), linalool (17.5%) and linalyl acetate (16.1%) as the major components of essential oil in Iranian population of *S. limbata*, while in Turkey samples α–pinene (11.2–24.3%), β–pinene (10.0–20.9%), and sabinene (14.6–17.4%) were the major compounds [10]. As observed, the type and amount of major essential oil compounds were completely different between these populations. Moreover, Nejadhabibvash et al. [13] studied

effect of ecological gradients on *S. limbata* anatomical traits. Several quantitative anatomical characteristics related to cuticle, collenchymas, pith, phloem, xylem, stomata frequency, epidermal number and stomata length/ width ratio differed among populations.

Findings of these investigations showed high level of infra–specific variability in S. *S. limbata. There are some reasons for morphological variations among these populations. It seems that genetic variations and ecological conditions of habitat are more important than others are. Several studies [18,20] have revealed that infra–specific genetic variation among populations leads to morphological difference. In addition, ecological factors have strong effect on morphological characteristics and through phenotype plasticity process lead to infraspecific morphological variations [21].limbata. There are some reasons for morphological variations among these populations. It seems that genetic variations and ecological conditions of habitat are more important than others are. Several studies [18,20] have revealed that infra–specific genetic variation among populations leads to morphological difference. In addition, ecological factors have strong effect on morphological characteristics and through phenotype plasticity process lead to infraspecific morphological variations [21].*

Fig. 5. CA–Joined plot of the studied populations with morphological characteristics. Abbreviations: blue symbols are population's number according to tables 1, calwi: calyx width, callen: calyx length, stle: style length, calshtele: calyx short teeth length, callontele: calyx long teeth length, callenwidrat: calyx length/width ratio, callotelewira: calyx long teeth length /width ratio, calshtelewira: calyx short teeth length /width ratio, petlenwira: petal length /width ratio.

According to UPGMA tree, PCA and PCO plots of morphological characteristics, we found four primitive phenotypes among these populations: phenotype no. 1 (population no. 1), phenotype no. 2 (population no. 5), phenotype no. 3 (populations no. 2 and 4), and phenotype no. 4 (including the rest populations). Each phenotype was characterized by special morphological characteristics.

S. limbata has large distribution range in Iran and grows in environmental heterogeneity habitats. Its populations achieve adaptations for living under different ecological conditions, therefore phenotype plasticity occurs in order to adaptation with ecological conditions.

Wherever the population's habitats are the same, the morphological characteristics of populations will be similar, regardless of geographical distance of populations. Studies of population on the pattern of variation in several plant species have revealed the existence of localized populations each adapted to the particular environmental conditions of their habitat [3].

Phenotype no.3 had two populations. The habitats of these populations were close together with similar ecological conditions; therefore, morphological similarity of them was very probable. Moreover, low geographical distance facilitates gene flow between populations and leads to lower differentiation. Hamrick and Godt [4] have suggested that higher inter–populational gene flow leads to lower differentiation among populations.

Phenotype no. 4 consisted of four populations. The distance between populations no. 3 and 6 was more than 700 km. In addition, it was ca 350 km between populations no. 7 and 8. Contrary with very large geographical distance these populations were clustered as a group. Moreover, in comparison to phenotype no. 3, phenotype no. 2 was less distant with phenotype no. 4, while according to UPGMA tree, PCO and PCA plots phenotype 3 was closer to phenotype no. 4 rather than phenotype no. 2.

These authors propose two main possible reasons for this similarity, despite the existence of a long geographical distance. The genetic similarity is the first possible case, Sheidai et al. [18] have suggested that the morphological similarity among populations may be due to genetic likeness, although, it is more important to know that phenotype and genotype are not necessarily tightly linked. For example, Liston [11] and Whitkus [24] have revealed high levels of genetic variations among morphologically similar populations of *Astragalus* sect. *Leptocarpi* and *Carex pachystach*, respectively.

What, unlike the long distance, has led to the similarity of populations is similarity of ecological conditions of habitats. This is well suited to the idea of ecological mosaic. Jain and Bradshaw [9] have suggested that if a plant species encounters a mosaic of habitats while expanding its range, the selective influences of each habitat act in turn upon the whole available morphologic difference.

Talebi et al. [20] studied morphological and ecological features of several populations of *Linum album* in Iran. They found that there is a strong relationship between morphology of populations and their ecological conditions. Wherever the ecological conditions were similar, the morphological characteristics of the plants were alike.

So, it seems that in the case of our studied populations, ecological factors have a very strong effect on population's similarity or difference. This condition creates morphological polymorphism among populations. Of course, this situation gives the species that has the genetic background to occupy a variety of environments and emergence of morphological polymorphism.

Conclusion

We evaluated inflorescence morphological characteristics among eight populations of *S. limbata*. Morphological traits highly differed among the populations and ANOVA test revealed significant difference among the populations. According to UPGMA tree, PCA and PCO plots, we found four primitive phenotypes, which were characterized by special morphological features. Two phenotypes were monotypic, while one phenotype consisted of two, and another one has four populations. The distribution domain of a species is not composed exactly identical ecological environments, but also a set of different environment with different ecological conditions. So species must adapt its phenotype structure in accordance with those conditions in order to be able to live under those conditions.

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