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Interspecific morpho-anatomical evaluation of diverse populations *Salvia macrosiphon* (Lamiaceae)

S. M. Talebi[1, 3], M. Samiei[1, 4], A. V. Matsyura[2]*

[1]Arak University, Shahid Beheshti Ave., P. O. Box 379, Arak, Markazi, 38156-8-8349, Iran

[2]Altai State University, Lenin St. 61, Barnaul, 656049 Russian Federation. E-mail: amatsyura@gmail.com; ORCID ID: https://orcid.org/0000-0001-9553-001X

[3] E-mail: seyedmehdi_talebi@yahoo.com; ORCID ID: https://orcid.org/0000-0002-9663-7350

[4] E-mail: maryam.samiee91@gmail.com; ORCID ID: https://orcid.org/0009-0002-6743-2310

**Corresponding author*

Keywords: anatomy, infraspecific diversity, morphometry, *Salvia*, taxonomy.

Summary. *Salvia macrosiphon* Boiss., a taxonomically complex species within the Lamiaceae family, is widely distributed across various regions of Iran. This study investigates the floral morphological characteristics and anatomical structures of the leaf midribs and petioles across 11 populations of this species. For morphological analysis, 10 flowering stems per population were examined, with 10 measurements taken for each floral trait. Anatomical studies involved the examination of five leaves and petioles per population. Data was analyzed using SPSS and PAST software. According to the ANOVA test, significant differences were detected among and within the examined characteristics. Furthermore, the PCA analysis explored that calyx length, calyx width, and calyx length width ratio were the more variable traits, which contained about 61 % of the total variations. A morphology-based UPGMA dendrogram divided the populations into two main branches. Anatomical studies revealed that all populations exhibited dorsi-ventral leaves, with notable variability in anatomical traits. PCA-biplot analysis identified distinct traits characterizing certain populations. The UPGMA clustering dendrogram based on anatomical data also revealed two distinct branches, with subgroups within each cluster. The clustering patterns based on morphological and anatomical characteristics were not consistent, highlighting differences in the morphological and anatomical responses of these populations to environmental conditions. Additionally, the observed diversity may be attributed to the existence of intraspecific taxa. These findings underscore the need for a re-evaluation of the infraspecific taxonomy of *S. macrosiphon* to better understand its taxonomic complexity and ecological adaptability.

Морфо-анатомическая межвидовая оценка различных популяций *Salvia macrosiphon* (Lamiaceae)

С. М. Талеби[1], М. Самиеи[1], А. В. Мацюра[2]

[1]Аракский университет, просп. Шахид Бехешти, а/я 379, Арак, Маркази, 38156-8-8349, Иран

[2]Алтайский государственный университет, пр. Ленина, д. 61, г. Барнаул, 656049, Россия

Ключевые слова: анатомия, внутривидовое разнообразие, морфометрия, таксономия, *Salvia*.

Аннотация. *Salvia macrosiphon* Boiss. является таксономически сложным видом семейства яснотковых (Lamiaceae), широко распространённым в различных регионах Ирана. В настоящем исследовании был проведён комплексный анализ морфологических характеристик цветков и анатомических структур срединных

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жилок листьев и черешков у 11 популяций данного вида. Для морфологического анализа из каждой популяции было отобрано по 10 цветущих побегов, при этом для каждого признака выполнялось по 10 измерений. Анатомические исследования включали изучение пяти листьев и черешков с каждой популяции. Собранные данные были обработаны с использованием статистических пакетов SPSS и PAST. Результаты выявили значимые различия ($P \leq 0{,}05$) в признаках цветков между популяциями. Согласно результатам ANOVA-теста, были выявлены значительные различия ($P \leq 0{,}01$) как между, так и внутри исследуемых характеристик. Кроме того, анализ главных компонент показал, что длина чашелистика, ширина чашелистика и отношение длины к ширине чашелистика являются наиболее изменчивыми признаками, на которые приходится около 61 % общей вариации. На основе морфологических данных дендрограмма, построенная методом UPGMA, разделила популяции на две основные группы. Анатомические исследования подтвердили дорсовентральное строение листьев во всех популяциях, при этом отмечалась значительная изменчивость анатомических признаков. PCA-биплот позволил выделить отличительные признаки, характерные для отдельных популяций. Дендрограмма кластеризации UPGMA, основанная на анатомических данных, также выявила две отдельные ветви с подгруппами внутри каждого кластера. Сравнение кластеризаций по морфологическим и анатомическим признакам выявило несоответствия, что указывает на различия в реакциях популяций на условия окружающей среды на морфологическом и анатомическом уровнях. Кроме того, наблюдаемое разнообразие может быть связано с наличием внутривидовых таксонов.

Introduction

The Lamiaceae family contains several genera: one of the largest and most important of them is *Salvia* L. This genus comprises of more than 1000 taxa and naturally grows in various regions of the world. Jamzad (2012) listed more than 60 taxa for *Salvia* in Iran. A large number of *Salvia* taxa explore different biological properties, such as antibacterial, antiviral, and antioxidant. Additionally, several *Salvia* species are economically important, and applied in different industries, including food, perfumery, and cosmetics (Kahnamoei et al., 2019; Balaei-Kahnamoei et al., 2021).

Salvia macrosiphon Boiss. is a perennial member of this genus with numerous populations that naturally grows across the country. This species is known as Marvak in Iran and explores different biological properties. For example, according to Hamedi et al. (2016), *S. macrosiphon* has been used as a diuretic, carminative and anti-flatulent medicinal plant in Iranian folk medicine. Additionally, several flavonoids, phenolics, and volatile compounds have been detected in this plant, with high biological activities, including antioxidant, antibacterial, cytotoxicity, and anticancer (Balaei-Kahnamoei et al., 2021).

This species has a problematic infraspecific taxonomy, and several infraspecific taxa were detected for *S. macrosiphon* in some previous literature (Talebi et al., 2022). For example, two varieties were reported for this species in "Flora Orientalis" (Boisser, 1879). Kharazian (2008) reported a new variety, *S. macrosiphon* var. *longiflora* Kharaz., for Iran with distinct morphological characters. Moreover, previous molecular genetic diversity study in Iranian populations of *S. macrosiphon* revealed the existence of high within-species genetic diversity (Talebi et al., 2022). Recently, trichome morphological investigation showed a high inferaspecific diversity among Iranian populations of this species (Talebi, Samiei, 2025). Meanwhile, no infraspecific taxa have been identified for this species in "Flora Iranica" (Recharger, 1984) and "Flora of Iran" (Jamzad, 2012).

Xie et al. (2024) suggested that morpho-anatomical characteristics of a leaf have great importance in plant taxonomy, species delimitation, and can successfully be used in designing evolutionary hypotheses and the reconstruction of phylogenetic dendrograms (Vilarinho et al., 2023).

Former examinations represented the importance of morphological and anatomical characteristics of leaves in the detection of infraspecific diversity in various *Salvia* species, for instance, *Salvia multicaulis* Vahl. (Talebi et al., 2017), *Salvia limbata* Benth. (Talebi et al., 2023), and other genera in Lamiaceae, including *Stachys inflata* (Talebi et al., 2014) and *Nepeta* spp. (Talebi, 2021). Due to the lack of infraspecific morpho-anatomical evaluation for *S. macrosiphon* in Iran, we conducted this study to find, how morphological and leaf anatomical characteristics vary at the infraspecific level. Therefore, this study aimed to examine (1) floral morphological diversity, (2) leaf midrib anatomical structure, and (3) petiole anatomical features in 11 Iranian populations of *Salvia macrosiphon*. As far as we could search, no similar study is available for *S. macrosiphon* in Iran, as well as the world.

Material and Methods

Plant Materials

Plant samples of 11 populations of *S. macrosiphon* were harvested from their natural localities in diverse regions of Iran (Table 1). These samples were identified according to descriptions provided in "Flora of Iran" (Jamzad, 2012), and the herbarium vouchers were prepared based on the traditional approaches in plant taxonomy.

Table 1. The localities' addresses and ecological characteristics of the *Salvia macrosiphon* populations

Code	Population name	Address	Latitude (N)	Longitude (E)	maximum temperature (°C)	Minimum temperature (°C)
1	'Saveh'	Markazi province, Saveh, 998 m	35°02'40"	50°35'49"	20.22	7.62
2	'Semnan	Semnan province, Semnan, 1060 m	35°22'67"	53°39'49"	21.37	9.31
3	'Mayamei'	Semnan province, Mayamei, 1025 m	36°24'48"	54°58'41"	14	2
4	'Sabzevar'	Khorasan Razavi, Sabzevar, 1146 m	36°21'52"	57°66'78"	19.25	10.79
5	'Mashhad'	Khorasan Razavi, Mashhad, 1146 m	36°29'72"	59°60'67"	19.59	10.98
6	'Takistan'	Qazvin province, Takistan, 1305	36°06'72"	49°69'61"	18.88	8.54
7	'Amir Kabir'	Markazi province, Amir Kabir new town, 1650 m	34°13'55"	50°06'28"	19.80	7.40
8	'Arak'	Markazi province, Arak, 1720 m	34°08'73"	49°70'22"	17.62	5.26
9	'Qadamgah'	Khorasan Razavi, Qadamgah, 1250 m	36°10'61"	59°06'08"	19.01	9.96
10	'Polor'	Mazandaran province, Polor, 2300 m	35°48'93"	52°12'85"	15.37	3.11
11	'Qazvin'	Qazvin, province, Avaj, 2100 m	35°42'96"	49°12'31"	18.92	8.56

Morphological Study

In total 9–11 flowering plants per population were selected for the morphometric analyses. Moreover, ten replications were performed per character measurement in each flowering plant. The examined morphological characteristics were calyx length, calyx width, calyx length/width ratio, calyx tooth length, calyx tooth width, spine length, calyx tooth length/width ratio, corrolla length, corolla width, and corolla length/width ratio. We performed the measurements using a digital caliper.

Anatomical Examination

The mature and intact leaves of 55 flowering stems (5 samples per population) from 11 populations of *S. macrosiphon* were immersed in the 70 % aldehyde-acetic acid-ethanol solution for 48 h. Then, samples were washed 3 times with distilled water and transferred to 70 % ethanol. The leaves and petioles sections were prepared manually and decolorized with 5 % sodium hypochlorite for 30 min. Finally, the sections were double-staned with carmine and methylen blue for 30 min and 5 s, respectively. The thin leaf and petiole sections were placed on clear microscopic slides and examined under a light microscopy (Olympus, CH2) at different magnifications. The evaluated anatomical characteristics of leaves and petioles were: leaves midrib shape, leaves ventral parenchyma cell layer no., leaves dorsal parenchyma cell layer no., petiole midrib shape, petiole midrib section no., petiole ventral parenchyma cell layer no., and petiole dorsal parenchyma cell layer no.

Statistical Analyses

The mean and standard deviations of the evaluated quantitative characteristics were calculated. The analysis of variance test (ANOVA) was performed to identify significant among and within population variations of quantitative traits. Additionally, Principal Component Analysis (PCA) was applied to detect more variable anatomical and morphological features. Pearson correlation coefficient analysis of bivariate data performed to identify significant correlations among the examined traits. All of these analyses performed using SPSS. ver. 16. Data was standardized and utilized for clustering analyses of these examined populations based on the morphological and anatomical features. In this regard, PAST software employed to construct dendrograms according to the unweighted pair-group method with the arithmetic average (UPGMA) method. Furthermore, Principal Component Analysis-biplot (PCA-biplot) was used to detect distinct traits of the examined populations.

Results

Morphological Study

The mean and standard deviations of the examined morphological traits were presented in Table 2. The largest and smallest lengths of calyx were detected in 'Mashhad' (19.5 mm) and 'Sabzevar' (13.5 mm) populations, respectively. The widest calyx (6.3 mm) belonged to 'Qadamgah' population, while, the narrowest one (3.4 mm) detected for 'Semnan' population. 'Saveh' and 'Qadamgah' populations had the widest (5.25 mm) and narrowest (2.55 mm) corolla, respectively. We found the largest corolla length (27.6 mm) in 'Takistan' population, but the smallest corolla (12.2 mm) observed in 'Meyamei' population. The largest (2 mm) and shortest (0.72 mm) spines were determined for 'Semnan' and 'Sabzevar' populations, respectively.

We detected several significant correlations between the examined quantitative morphological characteristics. For example, a significant positive correlation ($P < 0.01$, $r = 0.57$) was detected between calyx length and calyx width. Calyx length and width had significant positive correlations ($P<0.01$) with calyx tooth length and width. Corolla length had a positive significant correlation ($P < 0.01$, $r = 0.25$) with calyx width, however, we determined a negative significant correlation ($P < 0.01$, $r = -0.25$) between corolla length with calyx tooth width. Corolla width had a positive significant correlation with calyx width and calyx tooth ($P < 0.01$, $r = 0.31$).

According to the ANOVA test, significant differences ($P \leq 0.01$) were detected among and within the examined characteristics (Table 3). Furthermore, the PCA analysis (Table 4) explored that calyx length, calyx width, and calyx length width ratio were the more variable traits, which contained about 61 % of the total variations.

Table 2. The quantitative morphological characteristics of the *S. macrosiphon* populations examined

Population/traits		Calyx length	Calyx width	Calyx length/ width ratio	Corolla length/ width ratio	Corolla width	Corrolla length	Spine length	Calyx tooth lenght	Calyx tooth width	Calyx tooth length/ width ratio
'Saveh'	Mean	17.4	4.30	4.11	4.35	5.25	22.2	1.75	5.2	1.6	1.24
	SD	2.72	0.42	0.83	1.03	1.36	5.05	0.42	0.95	0.46	0.85
'Semnan'	Mean	14.3	3.4	4.27	7.62	3.3	24.2	2.00	2.80	1.3	3.88
	SD	1.16	0.39	0.52	1.72	0.71	21.40	0.53	0.63	0.35	0.93
'Meyamei'	Mean	14.6	3.44	4.28	7.33	2.94	12.2	0.94	3.83	1.89	2.06
	SD	1.02	0.46	0.41	2.03	0.3	4.68	0.17	0.71	0.33	0.38
'Sabzevar'	Mean	13.5	3.55	3.82	7.69	2.63	19.5	0.72	4.05	1.36	4.75
	SD	0.76	0.42	0.32	2.0	0.5	2.56	0.26	0.57	0.32	2.59
'Mashhad'	Mean	19.5	5.40	3.54	7.78	2.85	21.3	1.80	6.50	2.15	2.83
	StD	86/1	0.57	0.34	1.95	0.53	1.34	0.26	0.94	0.34	0.62
'Takistan'	Mean	18.4	5.45	3.40	6.06	4.65	27.6	1.20	4.75	2.05	2.35
	SD	1.49	0.64	0.4	1.52	0.53	4.14	0.26	0.92	0.44	0.42
'Amir Kabir'	Mean	16.00	5.35	3.22	6.20	4.65	26.6	1.70	4.55	2. 40	2.18
	SD	1.09	1.92	0.88	2.22	1.62	5.64	0.63	1.82	1.07	1.13
'Arak'	Mean	16.01	4.55	3.63	6.35	4.15	25.7	1.07	5.75	2.10	2.79
	SD	1.84	0.60	0.38	1.32	0.85	3.65	0.32	0.92	0.32	0.59
'Qadamgah'	Mean	13.9	10.3	4.54	8.00	2.55	19.6	1.85	4.30	1.45	3.02
	SD	1.73	0.32	0.6	1.84	0.5	1.26	0.41	1.42	0.37	0.90
'Polor'	Mean	15.9	4.45	3.60	5.00	4.25	21.6	1.3	4.50	1.60	3.00
	SD	0.77	0.5	0.3	1.15	0.59	5-Aug	0.35	0.71	0.57	1.28
'Qazvin'	Mean	17.3	4.15	4.18	8.20	3.10	24.9	1.25	6.75	1.75	3.93
	SD	3.56	0.41	0.6	1.4	0.66	3.61	0.35	0.92	0.35	0.48

Table 3. Results of the one-way ANOVA test among the morphological traits of *S. macrosiphon* populations

Characteristic		Sum of Squares	DF	Mean Square	F	Sig.
Calyx length	Between Groups Within Groups Total	3.78 3.99 7.77	11 **108** 119	3.43 3.70	9.29	0.00
Calyx width	Between Groups Within Groups Total	7.38 5.57 1.30	11 108 119	6.7 **10.52**	1.30	0.00
Calyx length/ width ratio	Between Groups Within Groups Total	1.80 3.06 4.85	11 108 119	1.63 0.28	5.77	0.00
Calyx tooth length	Between Groups Within Groups Total	9.57 1.05 2.01	11 108 119	8.70 0.98	8.94	0.00
Calyx tooth width	Between Groups Within Groups Total	1.30 2.54 3.84	11 108 119	1.19 0.24	5.05	0.00
Spine length	Between Groups Within Groups Total	1.80 1.67 3.47	11 108 119	1.64 0.15	1.0.7	0.00
Calyx tooth length/width ratio	Between Groups Within Groups Total	9.48 1.30 2.25	11 108 119	8.61 1.20	7.16	0.00
Corolla length	Between Groups Within Groups Total	9.93 1.70 2.70	11 108 119	9.02 1.57	5.74	0.00
Corolla width	Between Groups Within Groups Total	9.05 7.24 1.63	11 108 119	8.23 0.67	1.23	0.00
Corolla length/ width ratio	Between Groups Within Groups Total	1.67 3.03 4.71	11 108 119	1.52 2.81	5.42	0.00

Table 4. Outputs of the PCA analysis among the quantitative morphological features of *S. macrosiphon* populations

Characteristic	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
Calyx length	0.03	30	0.31	0.03	0.31	0.31
Calyx width	0.01	17	0.50	0.01	0.18	0.50
Calyx length/width ratio	0.015	14	0.65	0.015	0.15	0.65
Calyx tooth length	0.010	10	0.75	0.01	0.10	0.75
Calyx tooth width	0.88	8	0.84	ns	ns	ns
Calyx tooth length/width ratio	0.75	7	0.91	ns	ns	ns
Spine length	0.41	4	0.95	ns	ns	ns
Corolla length	0.31	3	0.99	ns	ns	ns
Corolla width	0.04	4	0.99	ns	ns	ns
Corolla length/width ratio	0.03	3	1	ns	ns	ns

Note: ns – nonsignificant value.

Clustering Analysis

According to the morphology-based UPGMA tree (Fig. 1), the studied populations were divided into two main branches. The small branch was composed of three populations, including the 'Meyamei', 'Sabzevar', and 'Qadamgah' populations. The largest branch had two clusters: one cluster was small and contained 'Polor', 'Saveh', and 'Mashhad' populations; another cluster was divided into two groups. The Semnan and 'Qazvin' populations were placed together as a group, and the rest populations ('Takistan', 'Amir Kabir', and 'Arak') constituent another group.

Anatomical Investigations

The leaves and petioles anatomical traits were summarized (Table 5). All the populations had leaves with a dorsi-ventral structure. A thin layer of epidermis was observed on the dorsal and ventral sides of leaves, which consisted of a single layer of oval to rectangular cells. Moreover, a thin layer of cuticle covered the external surface of the epidermal cells. Additionally, a dense indumentum was observed on both leaf sides. A thin layer of collenchyma tissue was detected under the epidermis tissue that was composed of 1 to 2 cell layers.

Table 5. The leaves and petioles anatomical characteristics of the *S. macrosiphon* populations examined

Population name	leaves			petioles			
	Midrib shape	Ventral cell layer no.	Dorsal cell layer no.	Midrib shape	Midrib segment no.	Ventral cell layer no.	Dorsal cell layer no.
'Saveh'	Linear	9	12	Semicircular	8	14	10
'Semnan	Arc shape	12	8	Arc	4	12	9
'Meyamei'	Linear	10	11	Arc	9	14	12
'Sabzevar'	Linear	10	8	Arc	2	20	10
'Mashhad'	Linear-Arc	8	8	Arc	5	16	8
'Takistan'	Linear-Arc	8	8	Arc	5	16	8
'Amir Kabir	arc	7	9	Arc	1	24	11
'Arak'	Linear	13	11	Arc	1	13	10
"Qadamgah'	Linear	13	11	Arc	1	13	10
'Polor'	Linear	15	10	Arc	1	8	8
'Qazvin'	Linear	12	10	Arc	3	7	6

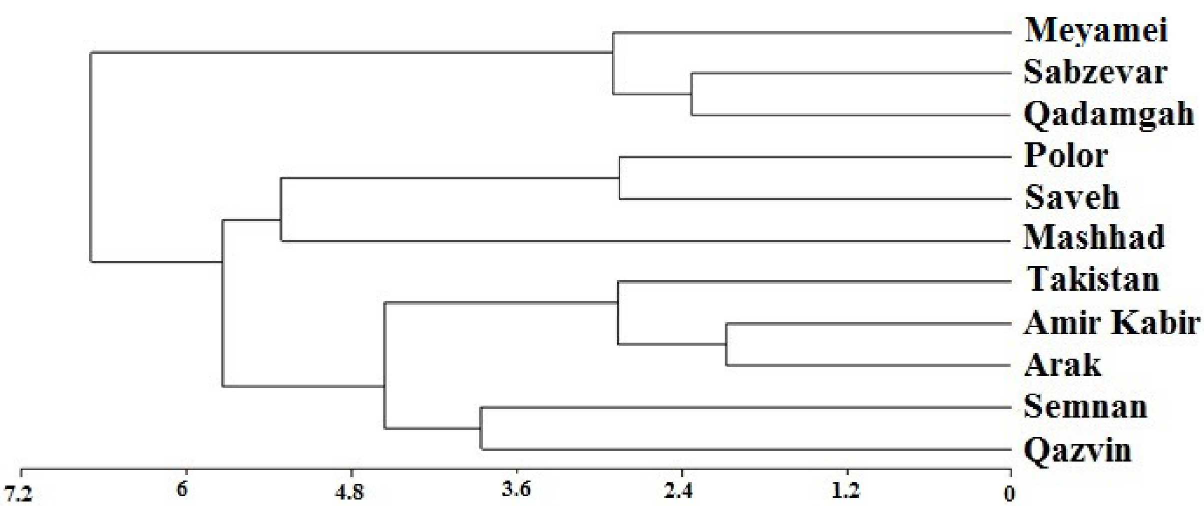

Fig. 1. The UPGMA dendrogram of the *S. macrosiphon* evaluated populations, based on morphological traits.

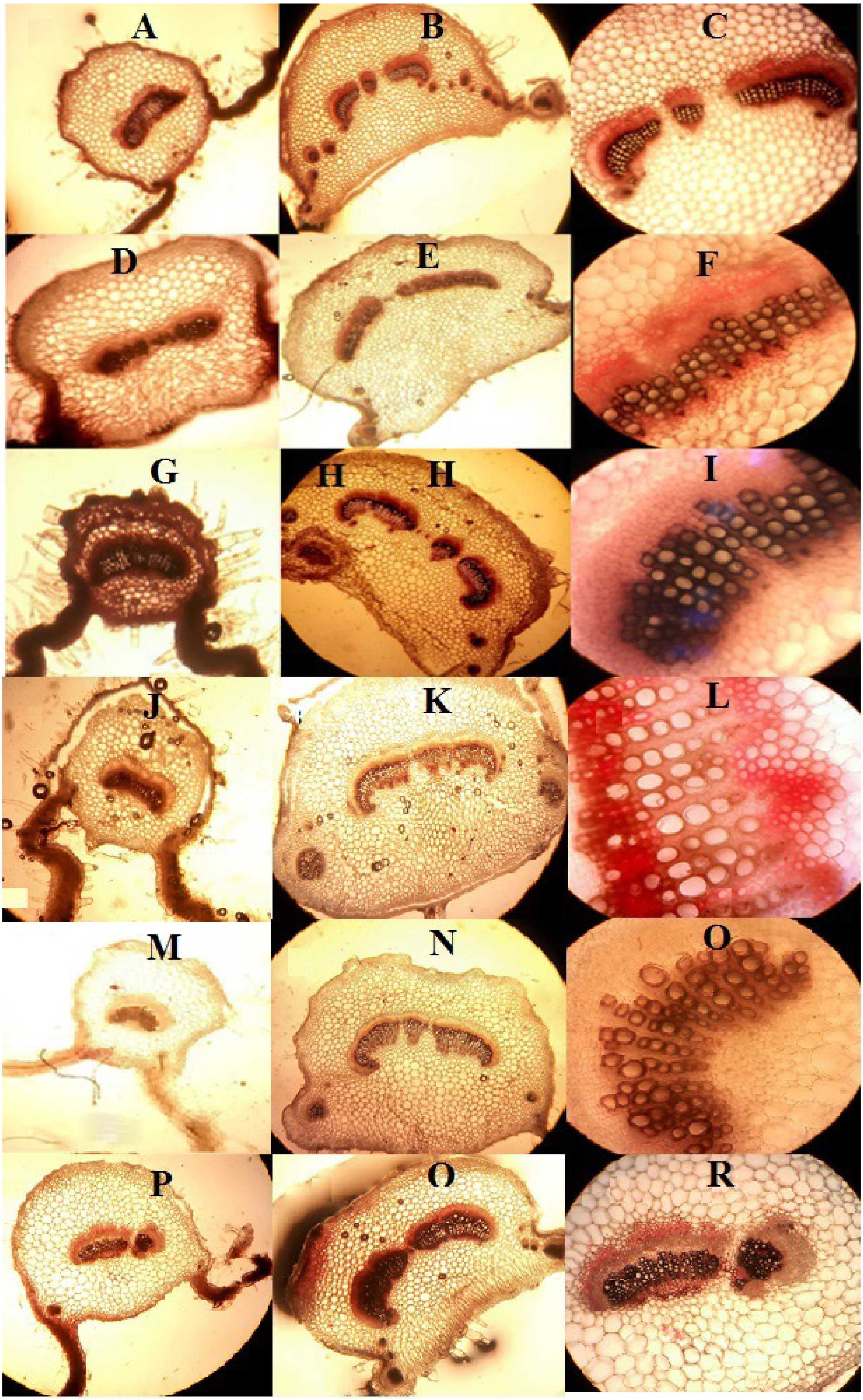

Fig. 2. Anatomical structure of the leaves, petiole, and vascular tissue of the *S. macrosiphon* populations examined: 'Meyamei' (A–C); 'Sabzevar' (D–F); 'Mashhad' (G–I); 'Takistan' (J–L); 'Amir Kabir' (M–O); 'Arak' (P–R).

Diverse layers no. of spherical to polygonal parenchyma cells were detected on the dorsal and ventral sides of the leaves midrib. In this regard, the largest (15) and smallest (6) ventral parenchyma cell layer no. were recorded for 'Polor' and 'Qadamgah' populations, respectively. However, the largest layer no. of parenchyma cells in the dorsal midrib belonged to 'Mashhad' population and the lowest detected for 'Qadamgah' population.

The leaf midrib vascular tissue shapes were linear ('Saveh', 'Meyamei', 'Sabzevar', 'Takistan', 'Arak', 'Ghadamgah', 'Qazvin, and 'Polor' populations), arc-shaped ('Semnan', 'Amir Kabir', and 'Qazvin' populations), and rarely linear-arc ('Mashhad' population). However, this tissue was one-segmented ('Saveh', 'Meyamei', 'Sabzevar', 'Mashhad', 'Takistan', 'Amir Kabir', 'Ghadamgah', and 'Polor' populations), two-segmented ('Semnan' and Arak populations), and rarely three-segmented (Qazvin population). There were nine and ten cell layers of phloem and xylem tissues on the midrib, respectively.

The main vascular tissue of petioles were in the shapes of linear ('Saveh' and 'Polor' populations), semicircular ('Semnan' and 'Ghadamgah' populations), and arc-shaped (in the rest populations). Furthermore, the main vascular tissue was one-segmented ('Takistan' population), two-segmented ('Ghadamgah', 'Arak', 'Amir Kabir', and 'Sabzevar' populations), three-segmented ('Saveh', 'Mashhad', and 'Qazvin' populations), and multi-segmented (the rest populations, see Fig. 2). The largest (24) and smallest (7) ventral parenchyma cell layer no detected for the 'Amir Kabir' and 'Qazvin' populations, respectively. Moreover, we detected the smallest layer no. of dorsal parenchyma cell for the 'Qazvin' population', and the largest one belonged to the 'Takistan' population.

The PCA analysis of anatomical features explored the first and second components, including 63.76 % and 19.32 % of the total variations, respectively. Therefore, we employed them for the PCA-biplot analysis, which explored that some of the evaluated populations had distinct anatomical characteristics. For example, the 'Polor' and 'Meyamei' populations were characterized by leaves ventral parenchyma cell layer no. and petiole midrib section no., respectively. Leaves midrib shape was a distinct trait for Mashhad population (Fig. 3).

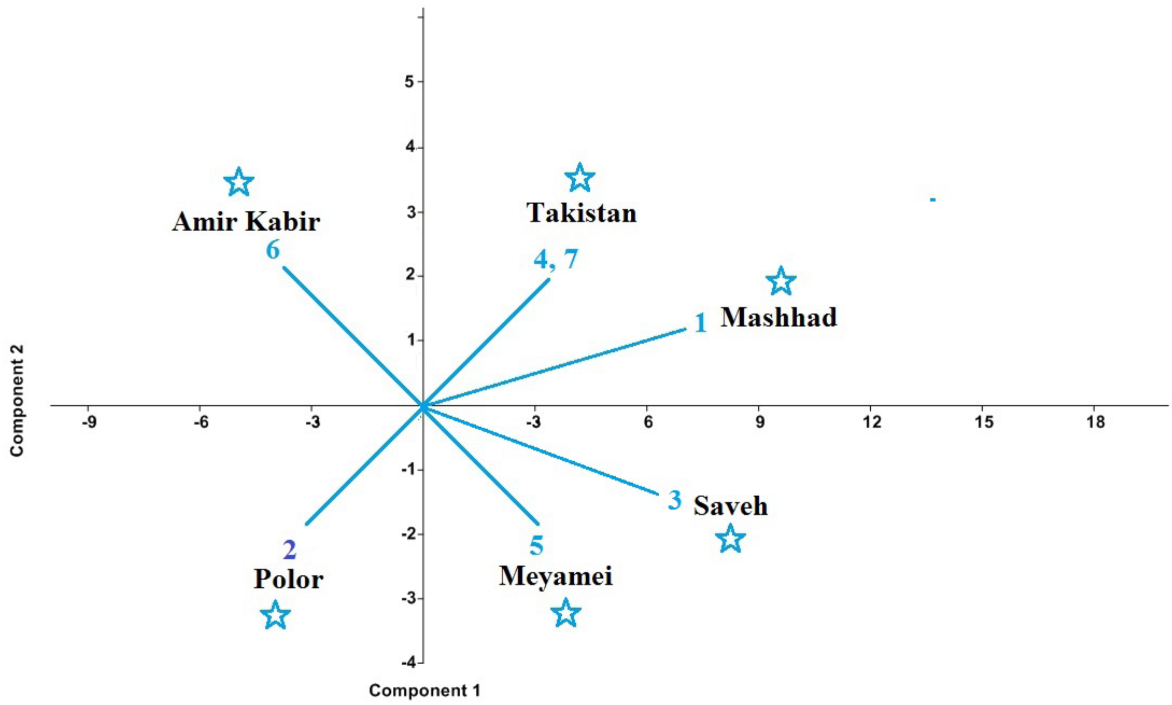

Fig. 3. PCA-biplot of the *S. macrosiphon* evaluated populations and the examined morphological traits. Abbreviation: 1 – leaf midrib shape; 2 – leaf ventral parenchyma cell layer no.; 3 – leaf dorsal parenchyma cell layer no.; 4 – petiole midrib shape; 5 – petiole midrib section no.; 6 – petiole ventral parenchyma cell layer no.; 7 – petiole dorsal parenchyma cell layer no.

The UPGMA clustering, based on the anatomical traits, revealed the presence of two main branches (with a bootstrapping support of 100 %): the smaller one consisted of three populations ('Amir Kabir', 'Takistan', and 'Sabzevar'), another one was divided into two clusters, and each cluster contained four populations. In this sense, 'Polor', 'Qazvin', 'Semnan', and 'Arak' populations were grouped as a cluster, while the rest populations ('Qadamgah', Meyamei, 'Saveh', and 'Mashhad') were placed in another cluster (Fig. 4).

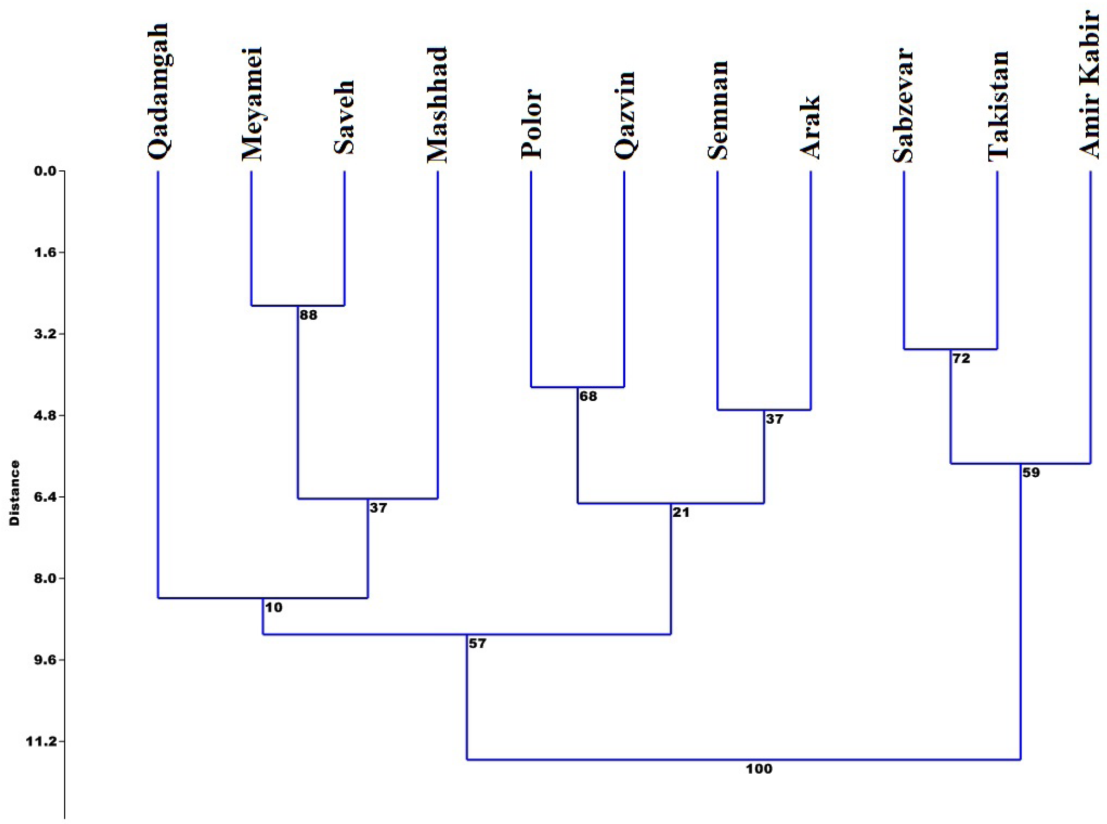

Fig. 4. The UPGMA clustering of the *Salvia macrosiphon* populations constructed on the anatomical traits.

Discussion

We detected high infraspecific diversity among the examined floral morphological characteristics of the evaluated populations. These variations explored the effect of environmental factors on the floral morphological traits. Strauss and Whittall (2006) believed that within species diversity of floral characteristics explores multiple and often conflicting selection pressures, also involving antagonistic conditions such as abiotic parameters. We think that altitudinal variations play a prominent role in the induction of floral morphological diversity among the populations. In this sense, more than 1300 m variations were detected between the lowest and highest altitudes of the population's habitats. According to previous studies (Körner, 2007), two categories of ecological variations were detected with altitudinal variations: some variations physically relate to altitude (a. s. l., m), such as atmospheric gas pressure, environmental temperature, and clear-sky turbidity. However, some others, for example, moisture, sunshine time, wind, length of seasons and geology are not generally altitude-specific. Therefore, the mentioned climatic variations strongly affected the morphological characteristics of *S. macrosiphon* populations, even the floral traits that are more stable than the vegetative ones.

Moreover, there were more than 2 and 10° variations in latitude and longitude gradients among the populations, respectively. In this regard, we detected nearly 18 and 11 °C variations between the maximum and minimum temperatures of harvested localities, respectively. These ecological variations strongly influence the floral features of *S. macrosiphon* populations. Paušič et al. (2022), who indicated temperature and precipitation regimes at the local scale significantly affect the dimensions of flowers, reported the same result.

Additionally, the induced stresses caused by the biotic pressures can affect the morphological characteristics, especially those of reproductive organs. As suggested by Weber et al. (2020), ecological biotic parameters can induce infraspecific variations in floral characteristics. Most flowering plants need animal's assistance for their pollination. However, climatic variations have prominent consequences for pollinator communities in each ecosystem, and alter pollinator-plant interactions (Tylianakis, Morris, 2017).

Aguirre et al. (2024) suggested that pollinator functional variation decreases with each increase in elevation. Each ecosystem has its fauna and the ecologically widespread species must adapt to their habitats to survive and find beneficial pollinators.

The pattern of clustering analyses, based on morphological traits, explored the existence of two morphotypes among the evaluated populations. In this regard, the 'Meyamei', 'Sabzevar', and 'Qadamgah' populations morphologically differed from the others and could be considered a morphotype group. These populations were harvested from the northeastern parts of the country. Therefore, the latitude has a strong effect on induction of the mentioned morphological divergence.

Although the Mashhad population is geographically very close to populations of this morphotype group, it was located in a separate cluster. Therefore, it seems that this population has a very different habitat than other surrounding populations, which has induced a morphology difference in it. In this regard, Paušič et al. (2019) suggested that environmental conditions, at the local scale, act as a strong driver of general floral diversity.

We evaluated petiole anatomical structure to use them as a taxonomic tool in the identification of infraspecific diversity in *S. macrosiphon*. Because former studies revealed that petiole anatomical traits have enough taxonomic potential to apply at species and infraspecific levels (Al-Dabbagh, 2022).

The shape of main midribs of petiole differed as arc, linear-arc, and semicircular. Therefore, these characteristics had a taxonomic importance at the infraspecific level. However, the arc shape was more frequent. It seems that the characteristic had an adaptive value for water availability in plants. Moreover, the main vascular tissues were divided into several segments in all the populations, except those for the 'Takistan', 'Amir Kabir', 'Arak', and 'Polor' populations that were one-segmented. Diversity in the vascular system of petioles was detected in numerous dicotyledonous taxa, and is considered a key anatomical trait in taxonomical treatments of diverse genera (Matias et al., 2007).

Anatomical population clustering revealed that environmental factors could strongly affect the internal structure of plants. For example, all the harvested populations of the northeast regions of the country were put close together, except for the 'Sabzevar' population that is placed far from others. Additionally, the 'Polor' and 'Qazvin' populations, which harvested from the mountainous areas placed close together. Liu and Zheng (2024) suggested that leaf is the main aerial organ of plants that plays a crucial role in growth and development of plants, due to its ability for photosynthesis and transpiration. Therefore, this organ plays a prominent role in the plant adaptation process to environmental stresses, and its anatomical structure can be utilized as a proxy for detection of variance.

However, all the infraspecific morphological and anatomical diversities did not directly relate to environmental conditions. Based on the former classic taxonomic treatments (Boisser, 1872; Parsa, 1950) different varieties have been detected for *S. macrosiphon*. Additionally, recent morphological (Karazian, 2008), molecular genetic diversity (Talebi et al., 2022) and trichomes morphology (Talebi, Samiei, 2025) on these populations explored high within species diversity in this species, which forces us to consider number of subspecies or varieties for *S. macrosiphon* in Iran.

Conclusion

Based on the distinct clustering observed in both morphological and anatomical data, the following groups are proposed as candidates for new intraspecific taxa: one comprising the populations from 'Meyamei', 'Sabzevar', and 'Qadamgah' populations, which form a distinct small branch in the morphological dendrogram, and another corresponding to populations placed separately in the anatomical dendrogram, including 'Sabzevar', 'Takistan', and 'Amir Kabir' populations. Especially, the 'Sabzevar' population which has been separated from others in both dendrograms. Additionally, this population placed separately in previous molecular genetic diversity study of the mentioned populations. To reinforce these taxonomic proposals, it is recommended investigation of leaves morphological characteristics and a comprehensive phylogenetic study using chloroplast (cpDNA), mitochondrial (mtDNA), along with ribosomal RNA (rRNA) genes must be conducted to reveal the evolutionary relationships between these populations, and validate the morphological and anatomical groupings.

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