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Research Article

Genetic diversity and essential oil composition of *Myrtus communis* L. from Lorestan Province, Iran: Implications for conservation and utilization

Somaye MOHEBI FARD[1], Maryam PEYVANDI[1]*,
Hossein ABBASPOUR[1], Ahmad MAJD[1], Sayeh JAFARI MARANDI[1]

*[1]Department of Biology, NT.C., Islamic Azad University, Tehran, Iran; somayeh.mohebifard@iau.ac.ir; maryampeyvandi@iau.ac.ir (*corresponding author); hosein.abbaspour@iau.ac.ir; a.majd@iau.ac.ir; s.jafari1377@iau.ac.ir*

Abstract

This study investigates the relationships between genetic diversity, environmental factors, and essential oil composition in *Myrtus communis* L., an evergreen medicinal shrub. Three populations in Lorestan Province, Iran (Cham Mord, Kaka Reza, Sepid Dasht) were analyzed using molecular and chemical techniques. Genetic diversity was assessed using Inter-Simple Sequence Repeat (ISSR) and Start Codon Targeted (SCoT) markers. ISSR (eight primers, 81 loci) showed highest diversity in Cham Mord (Shannon I = 0.26), while SCoT (eight primers, 71 loci) indicated Kaka Reza had highest diversity (I = 0.27). PCoA and cluster analysis under both markers showed Cham Mord was genetically distinct, with lowest genetic distance between Kaka Reza and Sepid Dasht (0.259) and highest between Cham Mord and Sepid Dasht (0.332). Gas Chromatography-Mass Spectrometry identified 37 essential oil compounds with significant variation. Cham Mord had higher α-pinene (72.05%) than Kaka Reza (22.45%) and Sepid Dasht (6.56%). Sepid Dasht showed highest oil yield, linked to lower altitude, higher phosphorus (13.8%) and organic carbon (1.24%), and lower salinity (EC = 0.24 ds m^{-1}). Kaka Reza, with higher salinity (EC = 0.41 ds m^{-1}) and sodium (19 mg l^{-1}), had the lowest yield. Essential oil-based clustering grouped Cham Mord and Kaka Reza, separating Sepid Dasht, matching SCoT patterns. Results reveal strong correlation between genetic diversity, oil profiles, and environmental factors including altitude, salinity, sodium, phosphorus, and organic carbon. Altitude inversely related to oil yield. Findings highlight local adaptation and phenotypic plasticity in *M. communis* chemodiversity, offering insights for conservation, sustainable use, and selection for medicinal and agricultural uses. Further studies on gene expression mechanisms of oil biosynthesis under varying environments are recommended.

Keywords: α-pinene; chemodiversity; essential oil yield; phenotypic plasticity

Introduction

Myrtus communis L., commonly known as myrtle, is a significant plant species recognized for its extensive use in traditional medicine and as a source of valuable essential oils applied in perfumery, cosmetics,

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and aromatherapy (Mishra, 2016; Shahbazian *et al.*, 2022). This evergreen shrub, indigenous to the Mediterranean basin and prevalent throughout the Middle East, demonstrates notable adaptability to a wide spectrum of environmental conditions. The leaves of *M. communis*, which are rich in volatile compounds, have historically been valued for their therapeutic properties, including potent antimicrobial, antioxidant, and anti-inflammatory activities (Petretto *et al.*, 2016; Quinn *et al.*, 2024). Crucially, the composition of these essential oils is highly dynamic, exhibiting significant variation influenced by factors such as genetic diversity, environmental conditions, and geographic origin, which contributes to the emergence of diverse chemotypes within the species (Azarafshan *et al.*, 2019; Mareri *et al.*, 2022; Rahman *et al.* 2023).

The study of genetic diversity in plant populations is fundamental to understanding their evolutionary trajectory and capacity to adapt to environmental stressors (Gupta and Bharalee, 2020). Genetic variations within plant genomes, whether subtle or pronounced, underpin a broad range of phenotypic expressions. These include variations in growth patterns and the biosynthesis of secondary metabolites, such as essential oils (Hazrati *et al.,* 2022). To effectively assess this genetic variation, molecular markers, specifically Inter-Simple Sequence Repeats (ISSR) and Start Codon Targeted (SCoT) markers, have become indispensable tools. These markers enable researchers to elucidate the population structures of plant species and unravel the intricate relationship between genetic makeup and environmental adaptation (Ghafouri and Rahimmalek, 2018; Mele *et al.,* 2019). Such molecular analyses are vital for informing effective conservation strategies and developing sustainable utilization plans for valuable plant resources.

Furthermore, environmental factors play a pivotal role in shaping the essential oil profile of aromatic plants like *M. communis*. Conditions such as soil characteristics, altitude, climate, and nutrient availability have been consistently shown to significantly influence both the yield and composition of essential oils (Mishra, 2016; Ahanger *et al.*, 2017; Sarmoum *et al.*, 2019). For instance, soil salinity has been linked to reduced essential oil yields in several plant species, while altitude variations often correlate with distinct changes in essential oil components. These environmental parameters can modulate the biochemical pathways responsible for essential oil synthesis, leading to chemotypes with varying biological activities and therapeutic efficacies. A thorough understanding of these influences is essential for optimizing cultivation practices and ensuring consistent product quality for both traditional and industrial applications. For a comprehensive ecological assessment, this study also considers temperature data, a key climatic variable influencing plant metabolism and secondary metabolite production.

This study focuses on investigating the genetic diversity and essential oil profiles of *M. communis* populations specifically within Lorestan Province, Iran. We analyzed populations from Cham Mord, Kaka Reza, and Sepid Dasht, integrating ISSR and SCoT molecular techniques with GC-MS chemical analyses. This integrated approach allows us to delineate the relationship between specific ecological factors, including temperature variations, and the observed variations in essential oil yield and composition. Moreover, we highlight how these combined dimensions of genetic and chemical diversity contribute to the overall adaptability and resilience of *M. communis* populations in response to environmental pressures.

The outcomes of this research are expected to deepen our understanding of *M. communis*' adaptive mechanisms. They will provide critical insights for the sustainable conservation and utilization of this important medicinal plant, informing the development of targeted breeding programs aimed at enhancing essential oil yield and quality. By integrating genetic and chemical data, we offer a more holistic perspective on the ecological and physiological factors driving phenotypic plasticity in *M. communis*, thereby facilitating more informed conservation practices and the sustainable exploitation of its valuable resources.

Material and Methods

According to the distribution map of the study habitats in Lorestan province, three regions, Kaka Reza, Sepid Dasht and Cham Mord, were selected. Various geographical features of the study areas were measured using the Global Positioning System (GPS). The slope, average altitude above sea level, slope percentage, main slope direction of the range and the study area were determined (Table 1). Samples were prepared from the soil of the areas and sent to the soil laboratory of the area and the texture type of the areas was determined (Table 2).

Table 1. Characteristics of three natural habitats of *M. communis* in Lorestan province

Habitats	Latitude	Longitude	Altitude above sea	Slope (%)	Slope direction
Kaka Reza	3716708	250399	1273	5	south
Cham Mord	3700909	775979	919	38	western
Sepid Dasht	3677541	294138	915	16	south

Table 2. Soil ccharacteristics of three habitats

Habitats	EC ($ds\ m^{-1}$)	pH	N (%)	Na ($mg\ l^{-1}$)	K ($mg\ l^{-1}$)	P (%)	Organic carbon (%)	Soil texture
Kaka Reza	0.41	8.13	0.11	19	330	7.6	1.32	clay, loam
Cham Mord	0.22	7.89	0.01	11	140	1.4	0.17	loam
Sepid Dasht	0.24	7.82	0.1	9	190	13.8	1.24	loam

Sampling and extract the essential oil

In July, sampling of plant leaves was prepared in the Cham Mord, Kaka Reza and Sepid Dasht regions. The collected leaves were dried in the shade at room temperature (25 °C) and transferred to the laboratory. 40 g of each sample was distilled with 300 ml of distilled water for 3 hours using the Clevenger apparatus (the extraction time starts from the first drop). The essential oil was dehydrated with sodium sulfate and the pure essential oil was weighed and stored in dark glass containers at 4 °C in the refrigerator.

Quantitative and qualitative measurement of essential oil with GC-MS

A GC gas chromatograph Agilent model N 6890, equipped with a HPS column with a length of 30 m, an internal diameter of 250 µm and a static surface layer thickness of 0.25 µm was used. The oven temperature was increased from 45 °C to 250 °C at a rate of 5 °C per minute and then reached 280 °C at a rate of 20 °C per minute. Helium gas with an ionization energy of 70 electron volts was used.

Essential oil analysis

SPSS Ver. 22 was used for data analyzing. The mean values of three replicates and Standard Error of means were calculated. One-way ANOVA was used to determine the significance of the differences between treatments and the Duncan's multiple range tests ($p < 0.05$) were performed.

Molecular experiments

DNA extraction

Five leaves as a pooled sample (48 samples, Table 1) were used for each treatment. Total genomic DNA was extracted from fresh leaves using the CTAB method of Murray and Tompson (1980) with modification described by De la Rosa *et al.* (2002). Separation on Agarose gel 0.8% (w/v) was used for quality examination of the extracted DNA. DNA concentration was measured by Nano Drop.

To investigate the intraspecific variation of the genome structure, ISSR (8 primers) and SCoT (8 primers) molecular techniques were used (Table 3). Three populations and 5 individuals from each population were used to perform molecular studies. Leaves of each species and known population were created and examined. DNA was extracted from each sample by CTAB method (Doyle and Doyle, 1990). Separation on Agarose gel 0.8% (w/v) was used for quality examination of the extracted DNA. DNA concentration was measured by Nano Drop.

Table 3. ISSR and SCoT primers used in this study

ISSR PRIMER name	Sequence
807	(GA)8T
810	(AG)8T
823	(TC)8C
849	(GT)8YA
(AGC)5GA	-
(AGC)5GC	-
(AGC)5GG	-
(AGC)5GT	-
SCoT PRIMER name	sequence
S1	CAACAATGGCTACCACCA
S3	CAACAATGGCTACCACCG
S7	CAACAATGGTCACCACGG
S11	AAGCAATGGCTACCACCA
S14	ACGACATGGCGACCACGC
S17	ACCATGGCTACCACCGAG
S18	ACCATGGCTACCACCGCC
S20	ACCATGGCTACCACCGCG

PCRs Bio-Rad (T-100, USA) thermal cycler was run for 5 min initial denaturation step 94 °C, 40 cycles of 1 min at 94 °C, 1 min at 55 °C, 1:30 min at 72 °C and final extension step was 10 min at 72 °C. Agarose gel 2% (w/v) was used for visualized the extension products.

Genetic analysis

100 bp molecular size ladder (Lactase fermentase) was used for estimating the fragment size. Parameter like Nei's gene diversity (He), Shannon information index (I), number of effective alleles (Ne), percentage of polymorphism (P %) and Nei's genetic distance were calculated (Weising *et al.*, 2005; Freeland *et al.*, 2011).

Social relationships were obtained through PCoA changes and cluster analysis using Genalex ver.6 and PAST ver. 2.17 software. To use genetic names and genetic differences between and within populations, AMOVA analysis was performed using GenAlex. Version 6.4 software.

Results

Quantity and quality of essential oil

The results of the percentage and chemical compositions of essential oil, including the type and percentage of compounds identified in the essential oil (obtained from plants in the three studied regions), are shown in Figures 1 and 2. Significant differences in essential oil yield and composition (number of compounds identified) exist across the three study sites. Sepid Dasht exhibited the highest yield (5.7% and 29 compounds), while Kaka Reza (4.28% and 26 compound), and Cham Mord (5.1% 23 compound). Alpha-pinene concentration varied drastically (72% in Cham Mord, 22% in Kaka Reza, 7% in Sepid Dasht).

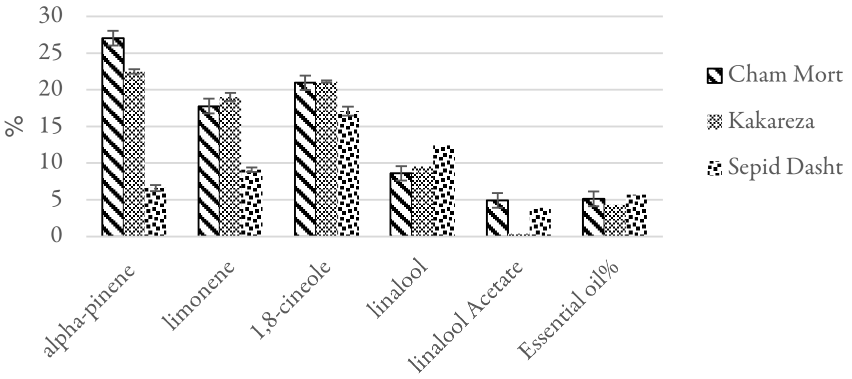

Figure 1. Main compounds obtained by GC-MS from plants in different regions. Bar = SE
Comparisons were made using Duncan's multiple range test (p ≤ 0.05). Common letters indicate no difference between means

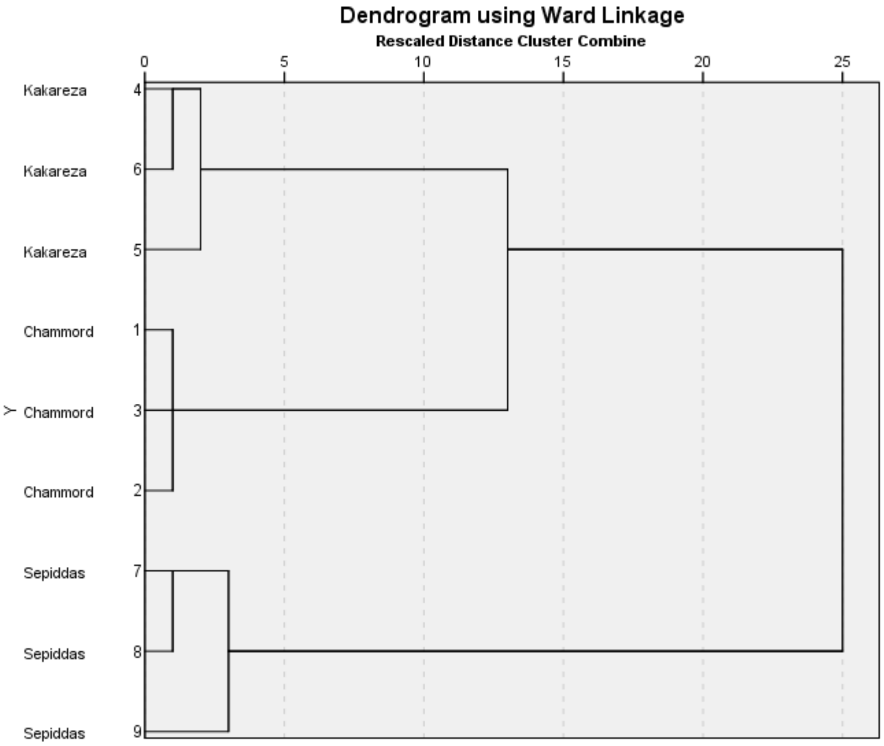

Figure 2. WARD cluster analysis of populations of different compositions of essential oil constituents

Analysis of variance showed that among the 37 identified compounds, apart from eight compounds (betaPinene, GammaTerpinene, CisLinaloolOxide, Terpinolene, Cymenene. LtransPinocarveol, MethylEugenol and Tridecanone), the difference in the percentage of other compounds among the different populations is significant (Figure 1). The largest difference in the essential oil was related to alpha-pinene. This compound was seen in the leaves of Cham Mord about 72%, in the Kaka Reza region about 22% and in the Sepid Dasht region about 7% (Table 4).

Table 4. Mean percentage (± SE) of compounds identified by GC-MS of essential oils in the leaves of the three *M. communis* populations

No.	Component	***M. communis*** **population**		
		Sepid Dasht	**Kaka Reza**	**Cham Mord**
1	Isobutyl isobutyrate	0.36 ± 0.02	1.1 ± 0.09	0.71 ± 0.07
2	alpha.-Thujene	0.0 ± 0.0	0.57 ± 0.10	0.3 ± 0.04
3	alpha.-Pinene	6.56 ± 0.29	22.45 ± 0.9	72.05 ± 0.04
4	beta.-Pinene	0.33 ± 0.1	0.5 ± 0.2	0.72 ± 0.06
5	Myrcene	0.0 ± 0.0	0.31 ± 0.2	0.03 ± 0.02
6	Delta-3-Carene	0.22 ± 0.03	0.0 ± 0.0	0.26 ± 0.4
7	p-Cymene	0.72 ± 0.10	0.03 ± 0.0	0.63 ± 0.08
8	Limonene	9.15 ± 0.09	19.1 ± 0.54	17.78 ± 0.3
9	1,8-Cineole	17.1 ± 0.07	21.11 ± 0.21	20.96 ± 0.76
10	beta.-Ocimene	0.0 ± 0.0	0.26 ± 0.04	0.0 ± 0.0
11	Gamma-Terpinene	0.0 ± 0.0	0.0 ± 0.0	0.21 ± 0.1
12	Cis-Linalool oxide	0.0 ± 0.0	0.04 ± 0.02	0.04 ± 0.04
13	Terpinolene	0.19 ± 0.02	0.17 ± 0.12	0.27 ± 0.05
14	Cymenene	0.0 ± 0.0	0.07 ± 0.02	0.08 ± 0.2
15	Dimethylstyrene	0.21 ± 0.03	0.0 ± 0.0	0.0 ± 0.0
16	Linalool	12.15 ± 1.12	9.54 ± 0.30	8.55 ± 0.34
17	L-trans-Pinocarveol	0.39 ± 0.02	0.17 ± 0.12	0.0 ± 0.0
18	Trans-Pinocarveol	0.0 ± 0.0	0.0 ± 0.0	0.61 ± 0.18
19	alpha-Terpineol	0.35 ± 0.17	0.5 ± 0.04	0.04 ± 0.03
20	Terpinen-4-ol	0.54 ± 0.08	0.04 ± 0.02	0.44 ± 0.03
21	Nerol	0.27 ± 0.14	4.51 ± 0.39	1.68 ± 0.18
22	Linalool Acetate	3.91 ± 0.17	0.34 ± 0.05	4.94 ± 0.11
23	Geraniol	3.25 ± 0.32	3.39 ± 0.29	0.28 ± 0.06
24	Pinocarveyl Acetate, trans	0.19 ± 0.05	0.02 ± 0.02	0.0 ± 0.0
25	Thymol	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.05
26	Carvacrol	0.67 ± 0.17	0.0 ± 0.0	0.0 ± 0.0
27	Methyl geranate	0.86 ± 0.09	0.22 ± 0.06	0.37 ± 0.02
28	Alpha-Terpinenyl Acetate	3.26 ± 0.04	0.19 ± 0.02	2.6 ± 0.17
29	Neryl Acetate	0.25 ± 0.07	2.24 ± 0.17	0.17 ± 0.03
30	Geranyl Acetate	2.41 ± 0.17	0.29 ± 0.04	1.14 ± 0.07
31	Methyl Eugenol	2.12 ± 0.25	2.0 ± 0.26	1.7 ± 0.22
32	Caryophyllene	6.62 ± 0.24	0.66 ± 0.11	0.8 ± 0.41
33	Alpha-Humulene	5.04 ± 0.17	0.58 ± 0.09	0.51 ± 0.15
34	2-Tridecanone	0.35 ± 0.23	0.0 ± 0.0	0.0 ± 0.0
35	Germacrene B	0.47 ± 0.22	0.0 ± 0.0	0.0 ± 0.0
36	Spathulenol	0.16 ± 0.04	0.0 ± 0.0	0.0 ± 0.0
37	Caryophyllene Oxide	1.14 ± 0.07	0.42 ± 0.24	0.29 ± 0.04

WARD cluster analysis based on essential oil constituents showed two main clusters, in which the Cham Mord population and the Kaka Reza population were located in two sub-clusters together, and the Sepid Dasht population was located in a separate cluster from the two other populations. This clustering indicates a greater similarity between the essential oil profiles of Cham Mord and Kaka Reza populations compared to Sepid Dasht. The distinct separation of Sepid Dasht suggests unique environmental influences or genetic makeup affecting its essential oil composition (Figure 2)

Results of genetic diversity with ISSR markers

The eight primers of the studied ISSR markers produced a total of 81 loci. The largest locus was 2800 bp, which was observed by the primer AGC5GC, and the smallest locus was 150 bp, which was observed by the same primer. Among the primers studied, the primer AGC5GC produced 21 loci, and primers 807 and 849 produced the least number of loci with 5 loci. The highest level of Shannon index (0.26), expected heterozygosity (0.18), and genetic polymorphism (46%) was in the Cham Mord population, and the lowest values of these indices were in the Sepid Dasht control population with 0.15, 0.1, and 28%, respectively.

The genetic matrix of reed for the different plant populations used was prepared from the data obtained from the ISSR markers. The lowest genetic distance was between the populations of Kaka Reza and Sepid Dasht (0.259). While it was observed between the populations of Cham Mord and Sepid Dasht (0.332). The genetic distance of the populations of Cham Mord and Kaka Reza was also 0.281.

PCOA classification for the principal component and with Jaccard coefficient was used in the next space based on ISSR molecular markers, which were placed in 3 separate groups based on 3 cases of the study plant populations (diagram). The result of this classification was consistent with the genetic distance matrix of the study populations. Accordingly, the two populations of Kaka Reza and Sepid Dasht are located on one side of the plot and closer to each other than the population of Cham Mord, and the population of Cham Mord is located on the other side of the plot with a greater distance (Figure 3). This PCOA analysis visually confirms the genetic relatedness suggested by the cluster analysis and genetic distance matrix. The proximity of Kaka Reza and Sepid Dasht populations on the plot indicates a higher degree of genetic similarity between them, while the wider separation of Cham Mord suggests it is genetically more distinct.

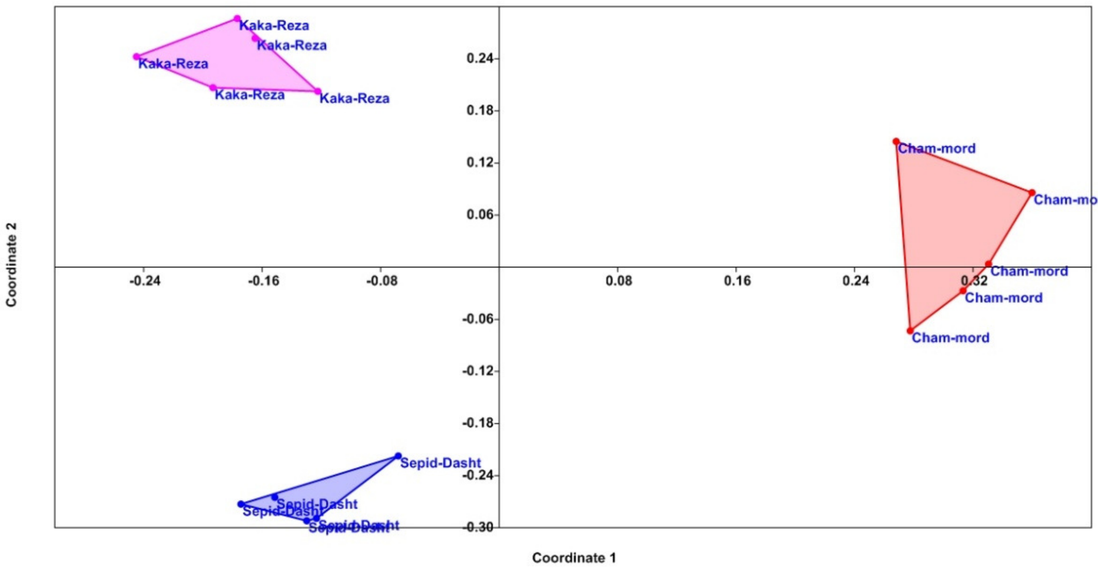

Figure 3. Classification of plant populations used in PCOA analysis based on Jaccard coefficient obtained from ISSR data

Results of statistical analysis of genetic diversity with SCoT markers

The eight SCoT marker primers studied produced a total of 71 loci. The largest locus was 2500 bp, which was observed by primers S14 and S18. The smallest locus was 100 bp, which was observed by primers S3 and S7. Among the studied primers, primer S14 produced 17 loci, and primers S1, S17, and S20 produced the least number of loci with 4 loci. The highest level of Shannon index (0.27), expected heterozygosity (0.19), and genetic polymorphism (0.46%) was observed in the Kaka Reza population and the lowest value of these indices was observed in the Sepid Dasht population with previous ranks of 0.19, 0.13, and 29.58%, respectively.

The genetic matrix of reed was prepared for the different plant populations used from the data obtained from the SCoT marker. The lowest genetic distance was between the Kaka Reza and Sepid Dasht populations (0.258). At the same time, it was observed between the Cham Mord and Kaka Reza populations (0.321). The genetic distance between the Cham Mord and Sepid Dasht populations was also 0.285.

The classification of the studied populations using the SCoT data showed that the three studied plant populations were placed into three distinct groups (Figure 4), which is consistent with the genetic distance matrix of the study populations. Accordingly, the two populations of Kaka Reza and Sepid Dasht are located on one side of the plot and closer to each other than the Cham Mord population, and the Cham Mord population is located on the other side of the plot with a greater distance. Similar to the ISSR analysis, the SCoT-based PCOA demonstrates a clear clustering pattern, reinforcing the findings that Kaka Reza and Sepid Dasht populations are more genetically related to each other than to the Cham Mord population. This consistency across different marker types strengthens the conclusion about the population structure.

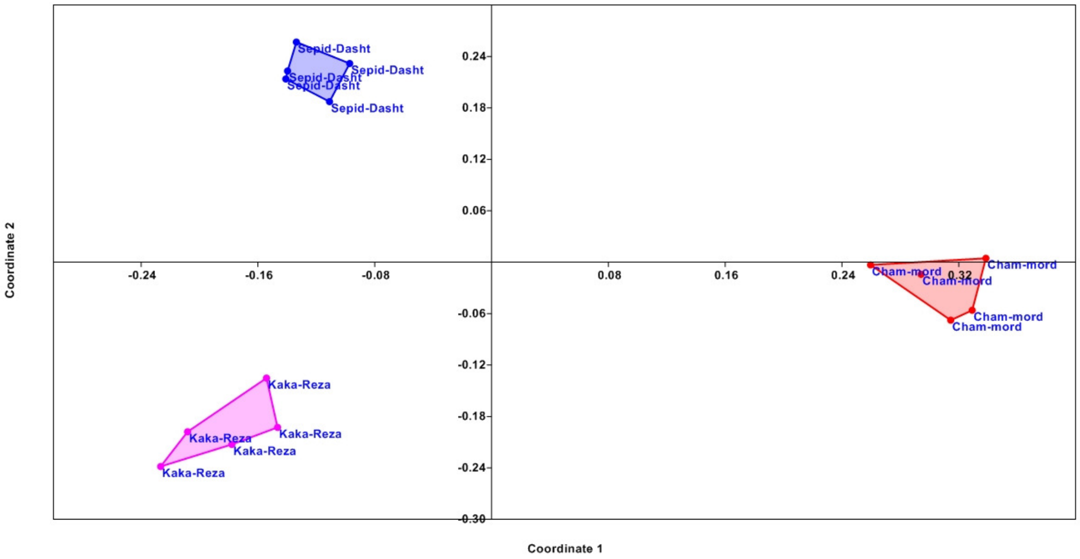

Figure 4. Classification of plant populations using PCOA analysis based on the Jaccard coefficient obtained from the SCoT data

In order to investigate the effect of combining data from ISSR and SCoT markers on the genetic divergence and diversity of populations, statistical analyses were also performed on the combination of loci obtained from these two markers. Based on the highest levels of Shannon index (0.24), expected heterozygosity (0.17), and polymorphicity (44.30%) in the Kaka Reza population and the lowest values of these indices were observed in the Sepid Dasht population, respectively, with 0.17, 0.12, and 29.53% (Table 5).

Table 5. Genetic variants (± SE) in the studied plant populations using ISSR and SCoT markers

Pop	N	Na	Ne	I	He	%P
Cham Mord	5.000	1.06 ± 0.071	1.312 ± 0.034	0.244 ± 0.025	0.169 ± 0.018	40.94
Sepid Dasht	5.000	0.913 ± 0.067	1.231 ± 0.032	0.179 ± 0.023	0.125 ± 0.017	29.53
Kaka Reza	5.000	1.18 ± 0.067	1.302 ± 0.032	0.251 ± 0.024	0.171 ± 0.017	44.30

Ne = number of alleles; I = Shannon index; He = expected heterozygosity; P% = percentage of polymorphism

The Nei genetic matrix was created for the different plant populations using the combination of ISSR and SCoT markers. The lowest genetic distance between the Kaka Reza and Sepid Dasht populations was

(0.275). While between Cham Mord and Sepid Dasht populations (0.314) was observed. The genetic distance between Cham Mord and Kaka Reza populations was also 0.305.

A similarity matrix was created using coefficients such as Jaccard, Dice, SM (Simple Matching) data obtained from ISSR and SCoT markers. The best dendrogram that also matches the Nei genetic matrix was observed based on the simple matching coefficient in the WARD tree (Figure 5). Results showed the Cham Mord population is located in a separate cluster from the other two populations. This dendrogram, generated using a combination of ISSR and SCoT data and employing the simple matching coefficient with WARD method, visually represents the genetic relationships. The branching pattern clearly separates the Cham Mord population into its own cluster, indicating a distinct genetic identity compared to the Kaka Reza and Sepid Dasht populations, which are grouped more closely together. This aligns with the PCOA results and genetic distance calculations, reinforcing the observed population structure.

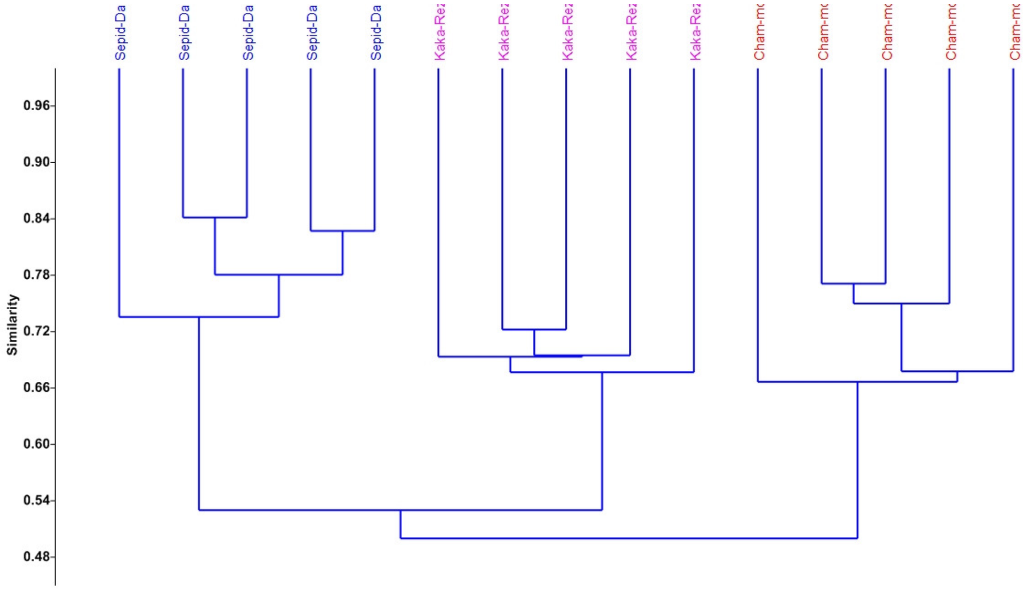

Figure 5. Ward dendrogram obtained from ISSR and SCoT data for the subject plant populations

Classification of plant populations using PCOA analysis based on the Jaccard coefficient obtained from the combined ISSR and SCoT data showed that the populations of Kaka Reza and Sepid Dasht are located on one side of the plot and closer to each other, while the Cham Mord population is located on the other side of the plot with a greater distance (Figure 6). This final PCOA plot, using combined ISSR and SCoT data, further solidifies the genetic structuring, reflecting a balance between adaptation to local conditions and genetic drift in isolated areas. The clustering of Kaka Reza and Sepid Dasht highlights their closer genetic relationship, contrasting with the more distinct genetic profile of Cham Mord. PCOA analysis allows us to visualize these complex relationships in a lower-dimensional space, complemented by the WARD method's optimal grouping based on similarity criteria. This visualization provides strong evidence for the genetic differentiation among the studied populations and the closer affinity between Kaka Reza and Sepid Dasht.

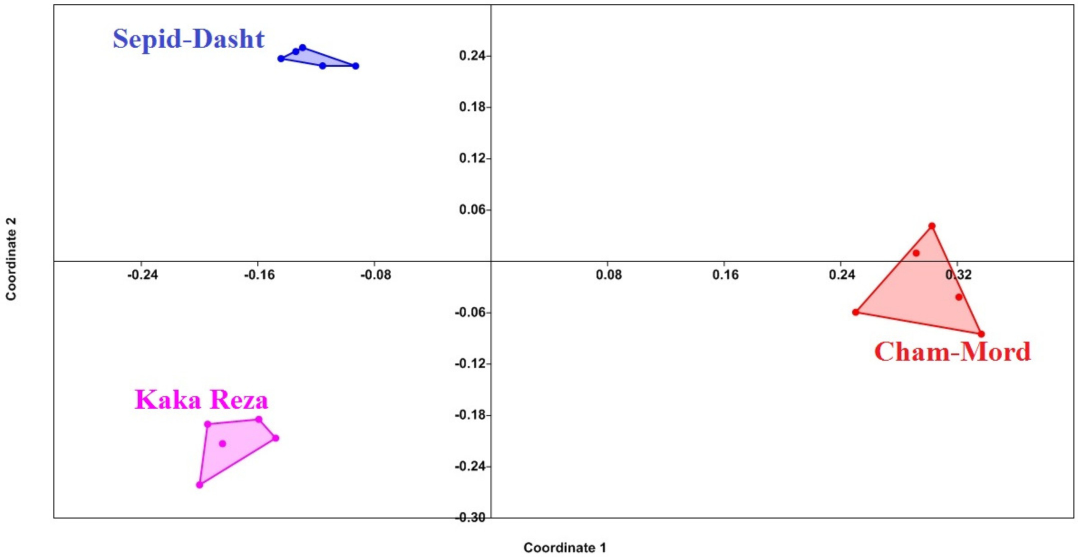

Figure 6. Classification of plant populations used in PCOA analysis based on the Jacquard coefficient obtained from ISSR and SCoT data

Discussion

The research underscores the substantial genetic diversity and essential oil variability of *M. communis L.*, highlighting the importance of ecological adaptations and the potential for conservation and breeding programs. The variations in essential oil profiles call for further exploration of the ecological influences on *M. communis L.* and its applications in traditional medicine.

This study explores the genetic diversity and essential oil composition of *M. communis* populations across three distinct regions of Lorestan Province, Iran: Sepid Dasht, Cham Mord, and Kaka Reza. The results reveal significant variations in both genetic attributes and phytochemical profiles among these populations, underscoring the vital role of environmental factors in shaping plant adaptability and their essential oil production.

Through GC-MS analysis, we identified that the quantity and quality of the chemical composition of essential oils of three populations are different. Significant differences in essential oil yield and composition exist across the three study habitats. The pronounced differences in alpha-pinene concentrations highlight how specific growing conditions in each habitat may favor the biosynthesis of certain metabolites, corroborating previous studies which linked soil type and nutrient availability to the production of secondary metabolites (Talebi, 2023).

Furthermore, the chemical variation among populations suggests that *M. communis* has adapted its metabolic pathways in response to local environmental factors. This adaptability enhances its pharmaceutical potential, as many of these compounds possess antimicrobial, anti-inflammatory, and antioxidant properties (Qaderi *et al.* 2023). Beyond alpha-pinene, other key compounds such as beta-pinene and gamma-terpinene also contribute to the overall essential oil profile and may play significant roles in the plant's defense mechanisms and medicinal properties (Al-Maharik *et al.*, 2023). The relative abundance of these compounds can vary significantly depending on phenological stages, environmental conditions and genetic factors, highlighting the complexity of essential oil biosynthesis (Petretto *et al.*, 2016; Hazrati *et al.*, 2022).

Environmental conditions, including such as salt and water stress (Saber *et al.* 2021; Rahimi *et al.* 2022), and climate (Zandalinas *et al.*, 2022), and soil properties (Ahanger *et al.*, 2017) are critical determinants of essential oil profiles in plants.

The relationship between soil characteristics and essential oil composition further underscores the complex interplay between ecology and phytochemistry. Soil texture (e.g., sandy, loamy, clay) affects water retention, aeration, and nutrient availability, directly impacting plant health and essential oil synthesis. High soil salinity negatively affects essential oil yield, consistent with prior research (Ahanger *et al.*, 2017; Talebi, 2023). This is likely due to salinity stress hindering nutrient uptake and water absorption. Results indicates that the higher essential oil yield in Sepid Dasht correlates with better soil quality (Low salinity, higher phosphorus, Nitrogen and organic carbon). This supports findings from Peng and Ng (2022), who noted that soil chemistry profoundly impacts the biosynthesis of essential oils, indicating the necessity for tailored cultivation practices that consider local soil conditions. Other research (Valkovszki *et al.*, 2023) supports the positive influence of nutrient-rich soils on essential oil production.

Moreover, the observed link between latitude, altitude and essential oil yield is noteworthy. The latitude of a location determines the climate (temperature, rainfall) and the length of day and night (photoperiod). These factors directly affect plant growth and metabolic processes. For example, at higher latitudes, plants might experience more intense sunlight during the summer months. Altitude is also a factor; higher altitudes often correlate with lower essential oil yields. Our findings indicate that regions with lower latitude and altitude (Sepid Dasht) exhibit higher essential oil production due to favorable growing conditions. Similar trends have been documented in other species, suggesting that altitude can influence metabolic rates and the biosynthetic pathways of essential oils (Walia *et al.*, 2020).

The application of molecular markers (ISSR and SCoT) revealed substantial genetic diversity across populations, with 81 ISSR loci and 71 SCoT loci identified. Cham Mord exhibited the highest genetic variability, while Sepid Dasht had significantly less diversity, which may reflect historical genetic bottlenecks or less favorable environmental conditions (Mele *et al.*, 2019). The genetic data, as revealed by ISSR and SCoT markers, provides insights into the genetic structure of the *M. communis* populations and its relationship to essential oil composition. The higher genetic diversity observed in Cham Mord may contribute to its distinct essential oil profile, particularly the high concentration of alpha-pinene. Conversely, the lower genetic diversity in Sepid Dasht, coupled with its unique environmental conditions, may explain its distinct essential oil composition. Further studies are needed to elucidate the specific genes and regulatory mechanisms that control essential oil biosynthesis in *M. communis* and how these are influenced by environmental factors (Mele *et al.*, 2019).

This pattern of genetic diversity is crucial for the resilience and adaptation of *M. communis* to changing environmental conditions. Higher genetic variability within a population provides a greater potential for adaptive responses to climate change, disease pressures, and habitat alterations. Our findings resonate with research by Mele *et al.* (2019), which highlight the necessity of conserving genetically diverse populations to sustain the ecological roles and medicinal applications of plant species.

The substantial genetic and chemical diversity observed in this study highlights the critical need for conservation strategies aimed at *M. communis*. Protecting populations with unique genetic traits and chemical profiles is essential for ensuring the sustainability of this species and its medicinal applications. Conservation efforts should be tailored to maintain the ecological integrity of habitats that support *M. communis*, recognizing that genetic diversity directly correlates with the species' resilience and adaptability.

Furthermore, fostering collaboration between local communities and conservationists can enhance the sustainable use of *M. communis*. Traditional knowledge regarding its applications in folk medicine can be integrated into modern conservation practices, ensuring the continued availability of this valuable resource for future generations.

Conclusion

In conclusion, this study provides an integrated overview of the relationships between genetic diversity, essential oil composition, and environmental factors in *M. communis* populations from Lorestan Province. The results reveal high genetic variability and distinct essential oil profiles, reflecting the species' adaptability to diverse habitats. Correlations between molecular markers, chemical constituents, and ecological conditions highlight the value of combining genetic and environmental approaches in conservation and sustainable use strategies. Protecting *M. communis* genetic resources is essential for its long-term survival and medicinal potential, while future studies should explore underlying molecular mechanisms to guide targeted breeding and cultivation for enhanced oil yield and resilience.

Authors' Contributions

Conceptualization: MP; Data curation: SMF, MP, HA; Formal analysis: MP, AM, SJ; Funding acquisition: -; Investigation: SMF; Methodology: MP; Project administration: MP; Resources: SMF,HA; Software: MP, HA; Supervision: MP; Validation: AM, SJ; Visualization: -; Roles/Writing - original draft: SMF, MP; Writing – review & editing: MP, HA.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Ahanger MA, Akram NA, Ahanger M, Alyemeni MN, Wijaya L, Ahmad P (2017). Plant responses to environmental stresses-from gene to biotechnology. AoB Plants 9(4):plx025. *https://doi.org/10.1093/aobpla/plx025*

Al Maharik N, Jaradat N, Al Hajj N, Jaber S (2023). *Myrtus communis* L.: Essential oil chemical composition, total phenols and flavonoids contents, antimicrobial, antioxidant, anticancer, and α-amylase inhibitory activity. Chemical and Biological Technologies in Agriculture 10:41. *https://doi.org/10.1186/s40538-023-00417-4*

Azarafshan M, Peyvandi M, Abbaspour H, Noormohammadi Z, Majd A (2019). The impact of UV-B radiation on antioxidant activity, essential oil composition and physiological factors of Pelargonium graveolens L'Hér. Acta Agriculturae Slovenica 113(2):211-219. *https://doi.org/10.14720/aas.2019.113.2.2*

De la Rosa R, James C, Tobutt KR (2002). Isolation and characterization of polymorphic microsatellite in olive (*Olea europaea* L.) and their transferability to other genera in the Oleaceae. Molecular Ecology Notes 2:265-267. *https://doi.org/10.1046/j.1471-8286.2002.00217.x*

Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Freeland J, Kirk H, Petersen S (2011). Molecular Ecology (2nd ed). Wiley-Blackwell, Chichester, UK. *https://doi.org/10.1002/9780470979365*

Ghafouri F, Rahimmalek M (2018). Genetic structure and variation in different Iranian myrtle (*Myrtus communis* L.) populations based on morphological, phytochemical and molecular markers. Industrial Crops and Products 123:489-499. *https://doi.org/10.1016/j.indcrop.2018.06.086*

Gupta S, Bharalee R (2020). Genetic diversity and population structure of *Houttuynia cordata* Thunb. in the Brahmaputra valley of North-East India. Plant Growth Regulation 91:434-442. *https://doi.org/10.1007/s11105-020-01260-9*

Hazrati S, Hosseini SJ, Ebadi MT, Nicola S (2022). Evolution of phytochemical variation in myrtle (*Myrtus communis* L.) organs during different phenological stages. Horticulturae 8(9):757. *https://doi.org/10.3390/horticulturae8090757*

Mareri L, Parrotta L, Cai G (2022). Environmental stress and plants. International Journal of Molecular Sciences 23:5416. *https://doi.org/10.3390/ijms23105416*

Mele C, Corona L, Melito S, Raggi L, Mulas M (2019). The genetic diversity of selections and wild populations of myrtle revealed by molecular markers in geographic contexts. Industrial Crops and Products 132:168-176. *https://doi.org/10.1016/j.indcrop.2019.02.018*

Mishra T (2016). Climate change and production of secondary metabolites in medicinal plants: A review. International Journal of Herbal Medicine 4(4):27-30.

Murray MG, Tompson WF (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research 8:4321-4325. *https://doi.org/10.1093/nar/8.19.4321*

Peng LC, Ng LT (2022). Impacts of nitrogen and phosphorus fertilization on biomass, polyphenol contents, and essential oil yield and composition of *Vitex negundo* Linn. Agriculture 12(6):859. *https://doi.org/10.3390/agriculture12060859*

Petretto GL, Maldini M, Addis R, Chessa M, Foddai M, Rourke JP, Pintore G (2016). Variability of chemical composition and antioxidant activity of essential oils between *Myrtus communis* var. Leucocarpa DC and var. Melanocarpa DC. Food Chemistry 197(Pt A):124-131. *https://doi.org/10.1016/j.foodchem.2015.10.056*

Qaderi MM, Martel AB, Strugnell CA (2023). Environmental factors regulate plant secondary metabolites. Plants 12:447. *https://doi.org/10.3390/plants12030447*

Quinn E, Ben Simchon E, Gorelick J, Oka Y, Frenkel O, Sionov E, … Shelef O (2024). Examination of genetic lines of *Myrtus communis* as potential sources of organic agricultural pest control agents. Heliyon 10(15):e35658. *https://doi.org/10.1016/j.heliyon.2024.e35658*

Rahimi A, Mohammadi MM, Moghaddam S, Heydarzadeh S, Gitari H (2022). Effects of stress modifier biostimulants on vegetative growth, nutrients, and antioxidants contents of garden thyme (*Thymus vulgaris* L.) under water deficit conditions. Journal of Plant Growth Regulation 41:2059-2072. *https://doi.org/10.1007/s00344-022-10604-6*

Rahman S, Iqbal M, Husen A (2023). Medicinal plants and abiotic stress: An overview. In: Husen A, Iqbal M (Eds). Medicinal Plants. Springer Nature, Singapore pp. 1-34. *https://doi.org/10.1007/978-981-19-5611-9_1*

Saber M, Harhar H, El Hattabi L, Zengin G, Bouyahya A, Tabyaoui M (2021). Chemical composition and antioxidant activities of essential oils and extracts from cones of *Tetraclinis articulata* (Vahl) Masters. International Journal of Secondary Metabolite 8:352-363. *https://doi.org/10.21448/ijsm.989436*

Sarmoum R, Haid S, Biche M, Djazouli Z, Zebib B, Merah O (2019). Effect of salinity and water stress on the essential oil components of rosemary (*Rosmarinus officinalis* L.). Agronomy 9(5):214. *https://doi.org/10.3390/agronomy9050214*

Shahbazian D, Karami A, Raouf Fard F, Eshghi S, Maggi F (2022). Essential oil variability of superior myrtle (*Myrtus communis* L.) accessions grown under the same conditions. Plants 11(22):3156. *https://doi.org/10.3390/plants11223156*

Talebi SM (2023). Changes in plant secondary metabolite profiles in response to environmental stresses. In: Ghorbanpour M, Shahid MA (Eds). Plant stress mitigators. Elsevier, Amsterdam pp. 325-339. *https://doi.org/10.1016/B978-0-323-89871-3.00018-5*

Valkovszki NJ, Szalóki T, Székely Á, Kun Á, Kolozsvári I, Szalókiné Zima I, Tavaszi Sárosi S, Jancsó M (2023). Influence of soil types on the morphology, yield, and essential oil composition of common sage (*Salvia officinalis* L.). Horticulturae 9(9):1037. *https://doi.org/10.3390/horticulturae9091037*

Walia S, Mukhia S, Bhatt V, Kumar R, Kumar R (2020). Variability in chemical composition and antimicrobial activity of *Tagetes minuta* L. essential oil collected from different locations of Himalaya. Industrial Crops and Products 150:112449. *https://doi.org/10.1016/j.indcrop.2020.112449*

Weising K, Nybom H, Pfenninger M, Wolff K, Kahl G (2005). DNA Fingerprinting. In: Weising K, Nybom H, Pfenninger M, Wolff K, Kahl G (Eds). Plants: Principles, methods, and applications. CRC Press *https://doi.org/10.1201/9781420040043*

Zandalinas SI, Balfagón D, Gómez-Cadenas A, Mittler R (2022). Plant responses to climate change: Metabolic changes under combined abiotic stresses. Journal of Experimental Botany 73:3339-3354. *https://doi.org/10.1093/jxb/erac073*

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