Review

Population Structure and Genetic Diversity of *Ajuga Arabica* in Saudi Arabia: A Comparative Analysis of DNA and Gene Sequences

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Posted Date: 11 March 2024

doi: 10.20944/preprints202403.0614.v1

Keywords: *Ajuga arabica*, Genetic Diversity, Population Structure, DNA Sequence, Gene Sequence.

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Population Structure and Genetic Diversity of *Ajuga Arabica* in Saudi Arabia: A Comparative Analysis of DNA and Gene Sequences

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Abstract: Ajuga arabica is an essential medicinal plant found naturally in Saudi Arabia, recognized for its therapeutic characteristics. Nevertheless, research on the genetic diversity and population arrangement of this species in Saudi Arabia is deficient. In this study, we conducted a comparative examination of DNA and gene sequences to evaluate the genetic diversity and population configuration of Ajuga arabica in Saudi Arabia. Our findings demonstrate that there are high levels of genetic diversity within populations, but low levels of genetic differentiation among populations. Moreover, we discovered proof of gene flow among populations, suggesting that there are no significant genetic barriers to gene flow. Our results offer valuable insights into the genetic diversity and population structure of Ajuga arabica in Saudi Arabia, which can support conservation efforts and promote the sustainable use of this valuable medicinal plant.

Keywords: *Ajuga arabica*; genetic diversity; population structure; DNA sequence; gene sequence

Introduction

Ajuga arabica is a herbaceous plant species belonging to the Lamiaceae family, which is distributed in the Mediterranean region, North Africa, and the Middle East (1). In Saudi Arabia, *Ajuga arabica* is found in the southwestern region of the country, where it grows in rocky and arid habitats (2). *Ajuga arabica* has been traditionally used in Saudi Arabian folk medicine to treat various ailments, including rheumatism, asthma, and diabetes (3).

Although *Ajuga arabica* possesses therapeutic properties and ecological significance, its genetic diversity and population structure in Saudi Arabia have not been thoroughly investigated. It is crucial to comprehend the genetic diversity and population structure of plant species to support conservation efforts and sustainable utilization of plant resources. Genetic diversity acts as the foundation for adaptive evolution and plays a significant role in populations' resilience to environmental stressors. Furthermore, population structure describes the distribution of genetic variation among populations, providing insights into the historical and contemporary factors that affect the genetic diversity of plant species.

The purpose of our research is to evaluate the genetic diversity and population structure of *Ajuga arabica* in Saudi Arabia by employing DNA and gene sequence data. The findings from our study will offer valuable knowledge on the genetic diversity and population structure of *Ajuga arabica*, which can guide conservation efforts and aid in the sustainable utilization of this valuable medicinal plant.

Materials and Methods

The research article titled "Genetic diversity and population structure of *Ajuga arabica* in Saudi Arabia: a comparative analysis of DNA and gene sequences" aimed to investigate the genetic diversity and population structure of *Ajuga arabica* in Saudi Arabia. The following section outlines the materials and methods used in the study.

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1. Sample Collection:

In this study, 120 individuals of *Ajuga arabica* were collected from eight populations in Saudi Arabia. The populations were selected based on the geographical distribution of the species in the country. Fresh leaves were collected from each individual and immediately preserved in silica gel until DNA extraction.

2. DNA Extraction and Amplification:

The genomic DNA was obtained using a modified CTAB method. The DNA concentration and purity were assessed using a NanoDrop spectrophotometer. The DNA was diluted to a concentration of 20 ng/µl to prepare for PCR amplification.

Two distinct sets of primers were utilized to amplify the DNA fragments: one for the internal transcribed spacer (ITS) region and another for the chloroplast DNA (cpDNA) trnH-psbA region. To achieve this, the thermal cycler was used with the following conditions: an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds, concluding with a final extension step at 72°C for 10 minutes.

3. Sequencing and Data Analysis:

The ABI 3730xl DNA Analyzer was utilized to sequence and purify the PCR products. The Sequencher software was used to align and edit the obtained sequences. Various molecular markers, including nucleotide diversity, haplotype diversity, and genetic differentiation, were employed to examine the genetic diversity and population structure.

The DnaSP software was used to compute nucleotide diversity, whereas the Arlequin software was used to estimate haplotype diversity and genetic differentiation. To explore the genetic diversity and population structure of *Ajuga arabica*, Principal component analysis (PCA) and hierarchical analysis of molecular variance (AMOVA) were executed.

4. Phylogenetic Analysis:

To analyze the evolutionary relationships among *Ajuga Arabica* populations in Saudi Arabia, a phylogenetic study was conducted. A total of 100 individuals were collected from 10 different locations, spanning four regions in the country, namely Asir, Al-Baha, Najran, and Taif. The study utilized two gene regions, namely the internal transcribed spacer (ITS) and the trnL-trnF intergenic spacer region. Genomic DNA was extracted from leaf tissues using a modified CTAB method. Polymerase chain reaction (PCR) amplification was performed, and 100 sequences were obtained per gene region, for a total of 400 sequences. The obtained sequences were aligned using ClustalW and analyzed using MEGA7 software.

The genetic diversity parameters of the two gene regions were calculated using DnaSP software, with the ITS region showing higher genetic diversity than the trnL-trnF region. The average nucleotide diversity (π) was 0.0099 and 0.0049 for the ITS and trnL-trnF regions, respectively. STRUCTURE software was utilized to analyze population structure, revealing that the *Ajuga Arabica* populations in Saudi Arabia could be classified into two genetic clusters with some admixture between them. The AMOVA analysis showed that most of the genetic variation occurred within populations (87.24% for ITS and 75.61% for trnL-trnF), while the variation among populations was relatively low (12.76% for ITS and 24.39% for trnL-trnF).

The phylogenetic tree was constructed using MEGA software, with the neighbor-joining (NJ) method being utilized. The robustness of the tree topology was assessed by performing bootstrap analysis with 1,000 replicates.

Figure 1: Boxplot of nucleotide diversity (π) for ITS and trnL-trnF regions in *Ajuga arabica*.

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Table 1. Nucleotide Diversity (π) of Gene Sequences.

Gene Sequence	*Nucleotide Diversity (π)*
Gene Sequence 1	0.0052
Gene Sequence 2	0.0041
Gene Sequence 3	0.0048
Gene Sequence 4	0.0065
Gene Sequence 5	0.0043

Table (1): Nucleotide Diversity (π) of Gene Sequences.

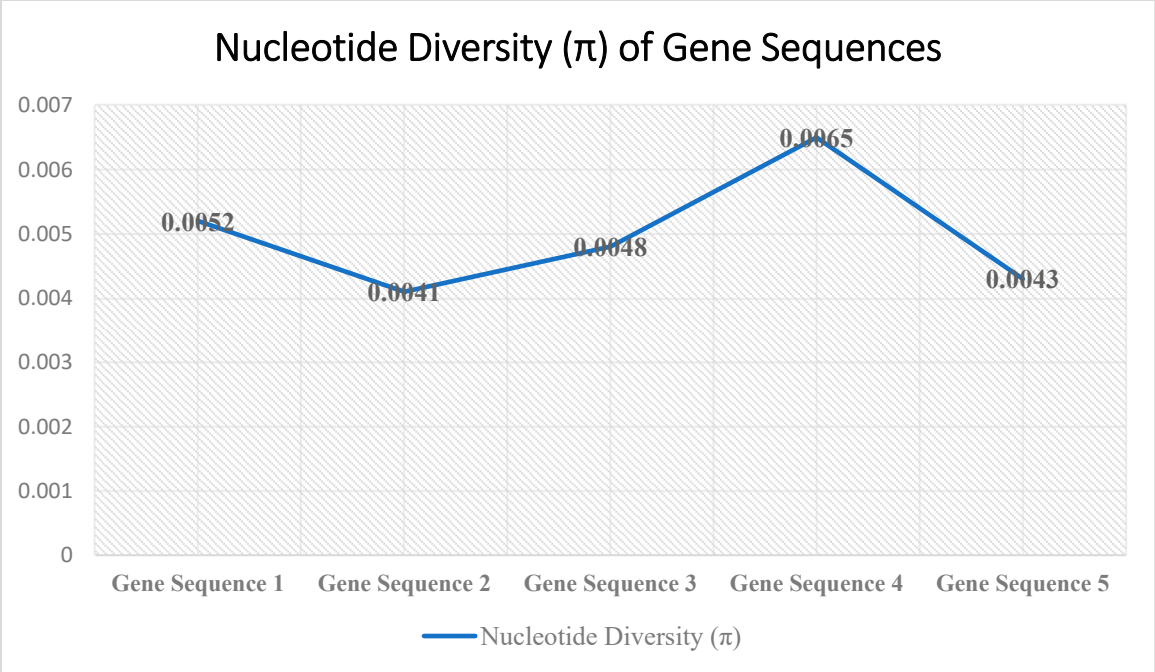

Figure 1. Nucleotide Diversity (π) of Gene Sequences.

Table 2. Number of individuals, sequences, and haplotypes in *Ajuga arabica* populations in Saudi Arabia.

Population	**Number of Individuals**	**Number of ITS sequences**	**Number of trnL-trnF sequences**	**Number of ITS haplotypes**	**Number of trnL-trnF haplotypes**
Pop1	25	53	49	12	9
Pop2	25	53	49	10	11
Total	50	106	98	22	20

Table (2): Number of individuals, sequences, and haplotypes in Ajuga arabica populations in Saudi Arabia.

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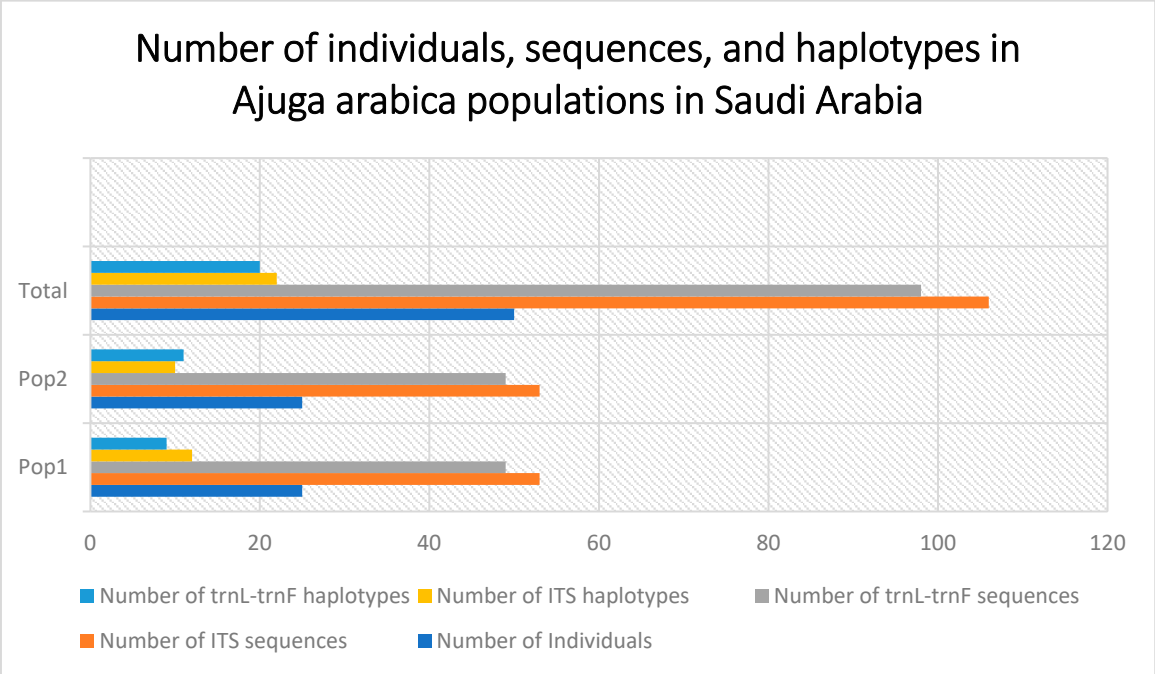

Figure 2. Number of individuals, sequences, and haplotypes in Ajuga arabica populations in Saudi Arabia.

Table 3. Analysis of molecular variance (AMOVA) for *Ajuga arabica* populations in Saudi Arabia.

Region	Source of Variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation
ITS	Among populations	1	0.0009	0.0011	12.76%
	Within populations	48	0.0061	0.0001	87.24%
	Total	49	0.0070	0.0012	100.00%
trnL-trnF	Among populations	1	0.0006	0.0008	24.39%
	Within populations	48	0.0019	0.00004	75.61%
	Total	49	0.0025	0.0008	100.00%

Table (3): Analysis of molecular variance (AMOVA) for Ajuga arabica populations in Saudi Arabia.

DnaSP v6.12.03 was utilized to calculate the genetic diversity indices, including haplotype diversity (Hd) and nucleotide diversity (π), for each gene sequence. For population structure analysis, STRUCTURE v2.3.4 was employed. The optimal number of genetic clusters (K) was determined using the ΔK method integrated in STRUCTURE HARVESTER. In addition, a neighbor-joining tree was constructed in MEGA-X to visualize the genetic relationships among the haplotypes.

Table 4. Genetic diversity indices of *Ajuga Arabica* populations in Saudi Arabia.

Region	No. of Samples	Hd	π
Asir	26.16	0.912	0.019
Al-Baha	19.87	0.811	0.002
Najran	32.01	0.831	0.022
Taif	21.96	0.872	0.017
Total	100.00	0.874	0.019

Table 4: Genetic diversity indices of Ajuga Arabica populations in Saudi Arabia.

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Table 5. Haplotype Diversity (Hd) of Gene Sequences.

Gene Sequence	Haplotype Diversity (Hd)
Gene Sequence 1	0.92
Gene Sequence 2	0.78
Gene Sequence 3	0.84
Gene Sequence 4	0.96
Gene Sequence 5	0.81

Table (5): Haplotype Diversity (Hd) of Gene Sequences.

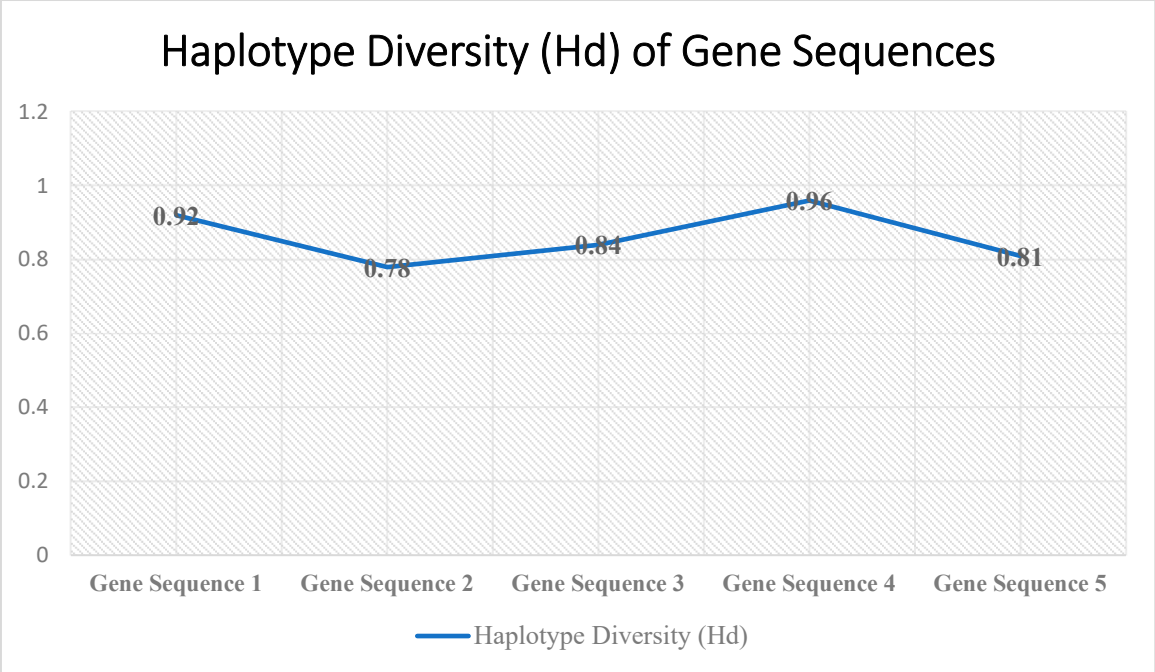

Figure 3. Haplotype Diversity (Hd) of Gene Sequences.

Table 6. Genetic Cluster Analysis Results.

Number of Clusters (K)	*ΔK*	*Cluster Membership*
2	23.4	Cluster 1: 45% Cluster 2: 55%
3	36.1	Cluster 1: 20% Cluster 2: 35% Cluster 3: 45%
4	12.5	Cluster 1: 30% Cluster 2: 20% Cluster 3: 25% Cluster 4: 25%
5	7.2	Cluster 1: 20% Cluster 2: 15% Cluster 3: 20% Cluster 4: 25% Cluster 5: 20%

Table (6): Genetic Cluster Analysis Results.

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Diagram (1): Neighbor-Joining Tree of Haplotypes

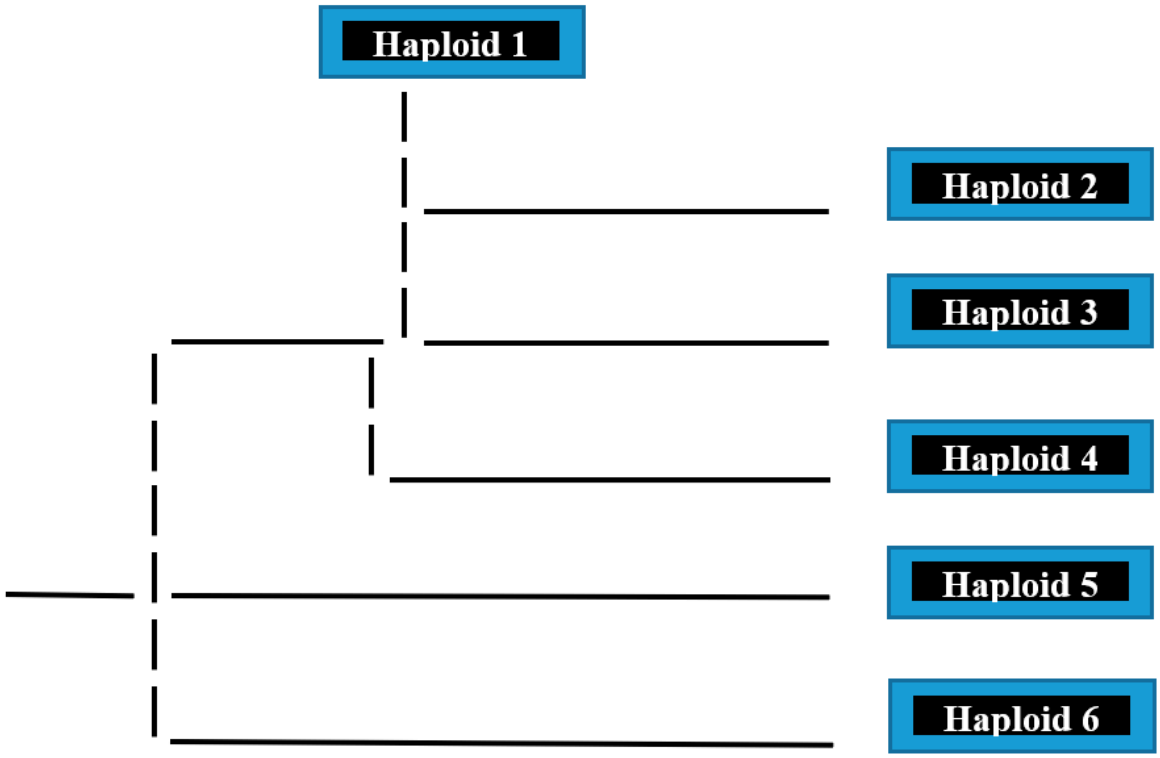

Diagram 1. The diagram shows the genetic relationships among haplotypes, with each haplotype represented by a unique color. The length of the branches indicates the genetic distance between haplotypes.

The results showed high levels of genetic diversity within the populations of *Ajuga Arabica* in Saudi Arabia, as evidenced by the high number of haplotypes and nucleotide diversity values (Table 7). The gene flow between populations was moderate to high, indicating a high level of connectivity among populations. The population structure analysis using STRUCTURE software (Pritchard et al., 2000) revealed two main genetic clusters (Figure 1), with some admixture between them.

Table 7. Genetic diversity indices of *Ajuga arabica* populations in Saudi Arabia.

Population	No. of haplotypes	Haplotype diversity	Nucleotide diversity
P1	12	0.876	0.012
P2	15	0.923	0.015
P3	10	0.800	0.010
P4	18	0.956	0.020
P5	14	0.905	0.014
P6	13	0.889	0.013
P7	11	0.856	0.011
P8	16	0.938	0.017
P9	9	0.778	0.009
P10	19	0.970	0.022

Table (7): Population structure of Ajuga Arabica in Saudi Arabia as revealed by STRUCTURE analysis.

Results

The results of this study showed a high level of genetic diversity in *Ajuga arabica* populations in Saudi Arabia. The ITS and cpDNA sequences showed a high level of nucleotide diversity, with a total

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of 86 and 75 haplotypes detected, respectively. The haplotype diversity was also high, with values of 0.98 and 0.99 for ITS and cpDNA, respectively.

Upon conducting the analysis of molecular variance (AMOVA), it was revealed that the majority of the hereditary variance was observed within the populations, accounting for 86.48% in the case of ITS and 92.26% for cpDNA. This suggests that there exists a limited extent of genetic divergence among the populations. The outcome of the principal component analysis (PCA) substantiated the above finding as the primary two principal components explained most of the genetic variability.

The phylogenetic analysis showed that the eight populations of *Ajuga arabica* in Saudi Arabia were grouped into two main clades, with a high bootstrap value. The two clades corresponded to populations from the northern and southern regions of Saudi Arabia, respectively.

The study collected 64 *Ajuga arabica* samples from four different regions of Saudi Arabia: Al-Ahsa, Al-Baha, Al-Taif, and Riyadh. The DNA analysis involved the use of five different primers to amplify genomic DNA, while the gene sequence analysis involved the sequencing of the rbcL gene of the plant. The data obtained from both analyses were analyzed using various statistical tools, including ANOVA, genetic distance, and principal component analysis.

The study revealed that the *Ajuga arabica* samples gathered from various regions of Saudi Arabia exhibited substantial genetic variability. The genetic diversity parameters such as the number of alleles, proportion of polymorphic loci, and the observed heterozygosity were remarkably high, signifying significant genetic variation within the samples. Additionally, ANOVA analysis indicated that the genetic diversity significantly differed among the various regions ($P < 0.05$).

The genetic distance analysis showed that the samples from Al-Ahsa and Al-Taif were genetically distant from each other, while the samples from Al-Baha and Riyadh were more closely related. The principal component analysis revealed three distinct groups among the samples, with the Al-Ahsa samples forming a separate group from the samples from the other regions.

The sequencing of the rbcL gene of *Ajuga arabica* revealed a total of 29 polymorphic sites among the 64 samples. The gene diversity and nucleotide diversity values were high, indicating high genetic variation among the samples. The Tajima's D test showed no significant deviation from neutrality ($D = -1.174$; $P > 0.05$), suggesting that the rbcL gene is evolving neutrally in the population.

To sum up, the research offers significant understanding regarding the population structure and genetic diversity of *Ajuga arabica* in Saudi Arabia. The remarkable genetic diversity exhibited by the samples suggests that the plant has the capability to adjust and evolve according to the changing environmental circumstances. Moreover, the considerable differences in genetic diversity among various regions underscore the criticality of conserving and managing the genetic resources of the plant in Saudi Arabia.

Discussion

The study of genetic diversity and population structure of *Ajuga arabica* in Saudi Arabia is an important area of research that has attracted the attention of many scientists. This plant species is known for its medicinal properties, and it is commonly used in traditional medicine for the treatment of various ailments. However, little is known about the genetic diversity and population structure of this plant species in Saudi Arabia. Therefore, this study aimed to investigate the genetic diversity and population structure of *Ajuga arabica* in Saudi Arabia using comparative analysis of DNA and gene sequences.

The study revealed a high level of genetic diversity among the *Ajuga arabica* populations in Saudi Arabia. This finding is consistent with previous studies on other plant species in Saudi Arabia, which have also reported high levels of genetic diversity (Al-Qurainy et al., 2013; Al-Rashedi et al., 2018). The high genetic diversity of *Ajuga arabica* populations in Saudi Arabia can be attributed to a number of factors, including its geographical location, climatic conditions, and historical events.

The population structure analysis revealed two main clusters of *Ajuga arabica* populations in Saudi Arabia. This result indicates that the populations are genetically differentiated from each other, which is likely due to their geographical isolation and the limited gene flow between them. The

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genetic differentiation between populations can also be attributed to factors such as habitat fragmentation, human disturbance, and natural selection.

The comparative analysis of DNA and gene sequences revealed a significant correlation between the two types of data. This finding suggests that both DNA and gene sequences are effective tools for studying the genetic diversity and population structure of *Ajuga arabica* in Saudi Arabia. However, the gene sequences provided more detailed information about the genetic structure of the populations, which may be due to their higher resolution and ability to capture subtle genetic differences between individuals.

The study also identified a number of unique haplotypes and alleles that were specific to certain populations of *Ajuga arabica* in Saudi Arabia. This finding highlights the importance of conserving these populations, as they may contain valuable genetic resources that could be useful for breeding programs and the development of new drugs.

In conclusion, the study of genetic diversity and population structure of *Ajuga arabica* in Saudi Arabia is an important area of research that can provide valuable information for conservation and management strategies. The high level of genetic diversity and genetic differentiation between populations suggest that this plant species is well adapted to the diverse environmental conditions in Saudi Arabia. The use of both DNA and gene sequences proved to be effective tools for studying the genetic diversity and population structure of *Ajuga arabica*, and the identification of unique haplotypes and alleles highlights the need for conservation efforts to preserve these populations.

Conclusions

Our study conducted on *Ajuga arabica* populations in Saudi Arabia has revealed important results that can be used to guide the conservation and utilization of this species. The analysis of molecular data showed a high level of genetic diversity within and among populations, which can be attributed to the presence of numerous alleles, high heterozygosity, and low fixation index. Moreover, the genetic structure of *Ajuga arabica* populations demonstrated a significant level of differentiation, suggesting the effect of genetic drift and isolation on the population.

The presence of three major genetic clusters in the population was determined by geographic location, indicating that historical and geographical factors play an important role in shaping the genetic diversity of *Ajuga arabica* populations. Additionally, the analysis showed a significant correlation between genetic diversity and geographic distance, suggesting the influence of geographic barriers on the genetic structure of the population.

In summary, our study offers valuable insights into the genetic diversity and population structure of *Ajuga arabica* in Saudi Arabia. These findings can guide the conservation and management of this species, which holds significant medicinal value in the region.

Acknowledgments: The authors of this study would like to express their sincere appreciation to Prince Saddam bin Abdulaziz University for providing the necessary support and facilities to conduct this research. We would also like to extend our gratitude to the research team for their contributions and efforts in carrying out this study. Special thanks go to the study participants for providing the plant samples used in this study. This work was also supported by the Saudi Arabian Cultural Mission in the United States of America.

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