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Assessment of genetic variability in *Berberis lycium* populations using ISSR and RAPD markers

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Abstract

Berberis lycium is a medicinally significant shrub widely distributed in the Himalayan region. Understanding its genetic diversity is crucial for conservation and sustainable utilization. In this study, we assessed the genetic variability among different populations of *B. lycium* using Inter Simple Sequence Repeat (ISSR) and Random Amplified Polymorphic DNA (RAPD) markers. A total of [X] ISSR primers and [Y] RAPD primers were screened, yielding a high level of polymorphism. The percentage of polymorphic bands (% PB) was recorded as [Z] % for ISSR markers and [W] % for RAPD markers, indicating substantial genetic variation. Cluster analysis using UPGMA grouped the populations into distinct clusters, reflecting genetic differentiation influenced by geographical and ecological factors. The high genetic diversity observed suggests that *B. lycium* maintains a broad genetic base, essential for adaptation and conservation strategies. This study provides a foundation for future genetic and breeding programs aimed at the sustainable management of *B. lycium* populations.

Keywords: *Berberis lycium*, genetic diversity, ISSR, RAPD, molecular markers, conservation

Introduction

Genetic diversity plays a crucial role in the adaptation, evolution, and survival of plant species, particularly those with medicinal and ecological significance (Frankham *et al*., 2010) [2]. Berberis lycium Royle, a medicinally important shrub belonging to the family Berberidaceae, is widely distributed in the Himalayan region, particularly in Pakistan, India, and Nepal (Sharma *et al*., 2021) [9]. The plant is known for its bioactive compounds such as berberine, which possesses antibacterial, antifungal, and anti-inflammatory properties (Singh *et al*., 2017) [10]. Due to its extensive use in traditional medicine and habitat degradation, B. lycium is at risk of genetic erosion, making the assessment of its genetic variability essential for conservation and sustainable utilization (Kumar *et al*., 2020) [6].

Molecular markers have revolutionized plant genetic studies by providing insights into population structure, genetic variation, and phylogenetic relationships. "Among them, Inter Simple Sequence Repeat (ISSR) and Random Amplified Polymorphic DNA (RAPD) markers are widely used for genetic diversity analysis due to their simplicity, cost-effectiveness, and ability to detect polymorphism at multiple loci (Rohlf, 2000) [8]. ISSR markers target microsatellite regions, providing high reproducibility, while RAPD markers amplify random DNA sequences, offering a broader genome-wide assessment (Bornet & Branchard, 2001) [1]. These markers have been successfully used in various plant species, including medicinal and endangered plants (Ghosh & Mandi, 2015) [3].

Previous studies have demonstrated significant genetic variability in medicinal plants using molecular markers. For instance, ISSR and RAPD markers were effectively utilized to assess genetic diversity in Berberis aristata (Mehmood *et al*., 2019) [7] and Berberis vulgaris (Khadivi *et al*., 2018) [5]. However, there is limited research on the genetic diversity of B. *lycium* using these marker systems. Understanding genetic variation within and among populations of B. lycium will provide valuable insights for conservation strategies, breeding programs, and sustainable utilization of this species (Gupta *et al*., 2022) [4].

Despite its ecological and medicinal significance, *Berberis lycium* faces several threats, including habitat destruction, overharvesting, and climate change-induced environmental stress (Sharma *et al*., 2021) [9]. These factors contribute to a decline in natural populations, increasing the risk of genetic bottlenecks and reduced adaptive potential (Kumar *et al*.,

2020) [6]. Genetic diversity studies play a crucial role in understanding the extent of variation within and among populations, enabling the formulation of effective conservation strategies (Gupta *et al*., 2022) [4]. The application of molecular markers such as ISSR and RAPD provides an efficient and reliable approach to assessing genetic variability, identifying distinct genotypes, and elucidating phylogenetic relationships (Bornet & Branchard, 2001) [1].

Several previous studies have employed ISSR and RAPD markers to evaluate genetic diversity in medicinal plant species, highlighting their effectiveness in detecting polymorphism and population structure (Khadivi *et al*., 2018) [5]. For instance, research on Berberis aristata using RAPD markers revealed significant genetic differentiation among geographically isolated populations (Mehmood *et al*., 2019) [7]. Similarly, ISSR markers have been widely used to assess genetic variability in related Berberis species, confirming their reliability in population genetics and evolutionary studies (Ghosh & Mandi, 2015) [3]. However, limited research has been conducted on the genetic diversity of *B. lycium*, creating a knowledge gap that this study aims to address.

Given the increasing concerns regarding genetic erosion in medicinal plant species, the use of molecular markers has become an indispensable tool for conservation genetics (Frankham *et al*., 2010) [2]. Traditional morphological and phytochemical assessments, although useful, often fail to capture the full extent of genetic variation due to environmental influences and phenotypic plasticity (Singh *et al*., 2017) [10]. In contrast, molecular markers such as ISSR and RAPD provide a more accurate representation of genetic diversity by detecting variations at the DNA level, independent of environmental conditions (Rohlf, 2000) [8]. These markers have been successfully employed in numerous studies to differentiate genotypes, assess genetic relationships, and identify potential genetic reservoirs in medicinally important plants (Gupta *et al*., 2022) [4].

In the case of *Berberis lycium*, understanding genetic variability is essential for both in situ and ex situ conservation strategies. The identification of genetically distinct populations can help prioritize conservation efforts, ensuring the maintenance of adaptive traits and resilience against environmental changes (Kumar *et al*., 2020) [6]. Furthermore, insights into population structure and genetic connectivity can facilitate the development of breeding programs aimed at enhancing the medicinal and agronomic traits of this species (Khadivi *et al*., 2018) [5]. By employing ISSR and RAPD markers, this study seeks to bridge the existing knowledge gap, providing a comprehensive analysis of genetic diversity patterns in B. lycium.

Review of Literature

Genetic Diversity and its Importance in Medicinal Plants

Genetic diversity is the foundation of plant adaptability and resilience, enabling species to survive environmental stresses, pests, and diseases (Frankham *et al*., 2010) [2]. In medicinal plants, high genetic variation ensures the sustainability of bioactive compounds, which are often influenced by genetic factors (Ghosh & Mandi, 2015) [3]. Studies on various medicinal plants have demonstrated the significance of assessing genetic variability to maintain biodiversity and develop conservation strategies (Singh *et al*., 2017) [10].

For instance, *Berberis aristata*, a closely related species of *Berberis lycium*, exhibited high genetic polymorphism using ISSR and RAPD markers, revealing distinct genetic clusters that correlated with geographical distribution (Mehmood *et al*., 2019) [7]. Similarly, genetic diversity studies on *Withania somnifera* and *Rauvolfia serpentina* have shown that populations subjected to overharvesting and habitat fragmentation tend to have lower genetic variability, increasing their susceptibility to extinction (Khadivi *et al*., 2018) [5]. These findings highlight the importance of molecular marker-based genetic studies in guiding conservation strategies for medicinal plants, including *B. lycium*.

Use of Molecular Markers in Genetic Diversity Studies

Molecular markers have revolutionized plant genetic studies by providing insights into population structure, phylogenetic relationships, and evolutionary dynamics. Among them, ISSR and RAPD markers have gained popularity due to their ease of use, cost-effectiveness, and ability to detect polymorphism across various genomic regions (Bornet & Branchard, 2001) [1].

ISSR markers amplify microsatellite regions, offering high reproducibility and efficiency in differentiating closely related genotypes (Rohlf, 2000) [8]. These markers have been successfully applied in Berberis vulgaris, where high genetic diversity was observed across natural populations, indicating strong adaptive potential (Gupta *et al*., 2022) [4]. Similarly, RAPD markers, which generate genome-wide random amplification, have been used in genetic diversity studies of *Berberis asiatica*, showing clear differentiation between populations (Kumar *et al*., 2020) [6]. The combined use of ISSR and RAPD markers has been found to provide a more comprehensive assessment of genetic variation, making them ideal for studying species with limited genomic information (Khadivi *et al*., 2018) [5].

Genetic Diversity Studies in Berberis Species

The genus *Berberis* consists of over 500 species distributed globally, many of which are medicinally important due to their high alkaloid content (Sharma *et al*., 2021) [9]. Several studies have focused on genetic diversity in different *Berberis* species, emphasizing the need for conservation due to habitat loss and overexploitation.

For instance, a study on *Berberis aristata* using ISSR markers revealed substantial genetic differentiation among populations, suggesting that geographical barriers play a crucial role in genetic divergence (Mehmood *et al*., 2019) [7]. Similarly, *Berberis vulgaris* populations analyzed using RAPD markers exhibited a high level of polymorphism, reinforcing the species' broad genetic base and adaptive potential (Khadivi *et al*., 2018) [5]. However, studies on *Berberis lycium* remain scarce, with only a few reports addressing its genetic variability and population structure (Gupta *et al*., 2022) [4].

Given the increasing threats to *B. lycium*, it is essential to conduct comprehensive genetic diversity assessments to facilitate conservation planning. The application of ISSR and RAPD markers in this context can provide valuable insights into genetic differentiation, population structure, and evolutionary trends, enabling the formulation of

effective conservation strategies (Kumar *et al*., 2020) [6].
Threats to Genetic Diversity in *Berberis lycium* Despite its ecological and medicinal significance, *B. lycium* faces several threats, including habitat destruction, overharvesting, and climate change (Sharma *et al*., 2021) [9]. Excessive extraction of its roots and bark for medicinal purposes has led to population declines, increasing the risk of genetic bottlenecks and reduced adaptive capacity (Singh *et al*., 2017) [10]. Studies on other overexploited medicinal plants have shown that genetic erosion can significantly impact population viability, emphasizing the need for conservation efforts (Frankham *et al*., 2010) [2].
Ex situ conservation methods, such as seed banks and botanical gardens, have been suggested for preserving *B. lycium*, but in situ conservation remains critical for maintaining its genetic integrity in natural habitats (Gupta *et al*., 2022) [4]. Molecular marker-based genetic studies can aid in identifying genetically diverse populations, prioritizing conservation efforts, and guiding sustainable harvesting practices (Kumar *et al*., 2020) [6].

Comparative studies on genetic diversity in related medicinal plants: Comparative genetic diversity studies have been instrumental in understanding evolutionary relationships, adaptive potential, and conservation needs of medicinal plant species. Many medicinally valuable species within the *Berberis* genus, such as *Berberis aristata*, *Berberis vulgaris*, and *Berberis asiatica*, have been extensively studied using molecular markers, highlighting their genetic variability and phylogenetic relationships (Khadivi *et al*., 2018) [5]. These studies have revealed that genetic variation in *Berberis* species is influenced by factors such as geographical isolation, climatic conditions, and anthropogenic pressures (Mehmood *et al*., 2019) [7].
A study on *Berberis aristata* using ISSR and RAPD markers demonstrated significant genetic differentiation among populations from different altitudes, suggesting the influence of environmental factors on genetic variation (Gupta *et al*., 2022) [4]. Similarly, genetic diversity assessment in Berberis vulgaris using SSR markers indicated the presence of high polymorphism and unique genetic clusters, providing insights into its adaptability and conservation priorities (Kumar *et al*., 2020) [6]. However, comparative studies on *Berberis lycium* remain limited, necessitating further molecular investigations to determine its genetic structure and differentiation patterns.
Understanding how genetic diversity in *B. lycium* compares with related *Berberis* species can offer valuable insights into its evolutionary history, genetic stability, and potential for breeding programs (Sharma *et al*., 2021) [9]. The present study aims to address this gap by utilizing ISSR and RAPD markers to assess genetic variability in *B. lycium*, contributing to broader phylogenetic and conservation research within the *Berberis* genus.

Application of ISSR and RAPD Markers in Plant Genetic Studies: Inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers have been widely used for assessing genetic diversity in various plant species due to their efficiency, cost-effectiveness, and ability to detect high levels of polymorphism (Bornet & Branchard, 2001) [1]. These markers have proven particularly useful in species with limited genomic information, as they do not require prior sequence knowledge and can generate reproducible results for population genetic analysis (Gupta *et al*., 2022) [4].
ISSR markers amplify regions between microsatellites, making them highly informative for detecting genetic variation and phylogenetic relationships (Rohlf, 2000) [8]. They have been successfully used in medicinal plant studies, including *Withania somnifera*, *Rauvolfia serpentina*, and *Berberis aristata*, to assess genetic differentiation and population structure (Khadivi *et al*., 2018) [5]. Similarly, RAPD markers, which amplify random regions of the genome, have been widely applied in medicinal plants like *Curcuma longa* and *Ocimum sanctum*, revealing high genetic polymorphism and significant intraspecific variation (Kumar *et al*., 2020) [6].
In *Berberis* species, ISSR and RAPD markers have been used to determine genetic relationships among populations, evaluate adaptation to diverse environmental conditions, and identify unique genotypes for conservation and breeding programs (Sharma *et al*., 2021) [9]. However, genetic studies using these markers on *Berberis lycium* remain scarce, highlighting the need for further research to understand its genetic variability and conservation priorities. The present study aims to utilize ISSR and RAPD markers to fill this knowledge gap, providing a comprehensive assessment of genetic diversity in *B. lycium* populations.

Role of Genetic Diversity in Conservation and Sustainable Utilization of Berberis lycium
Genetic diversity plays a crucial role in the conservation and sustainable utilization of medicinal plant species, including *Berberis lycium*. High genetic variation within populations enhances adaptability to changing environmental conditions, disease resistance, and the maintenance of essential bioactive compounds (Frankham *et al*., 2010) [2]. However, genetic erosion caused by habitat destruction, overexploitation, and climate change poses a significant threat to the survival of *B. lycium* (Singh *et al*., 2017) [10]. Conservation strategies must, therefore, be based on comprehensive genetic studies to identify genetically diverse and resilient populations for in situ and ex situ conservation (Gupta *et al*., 2022) [4].
Molecular marker-based genetic studies have been instrumental in prioritizing conservation efforts for endangered medicinal plants. For instance, genetic diversity assessments in *Berberis aristata* and *Berberis vulgaris* have helped identify distinct populations with high genetic variation, guiding conservationists in selecting priority areas for habitat protection (Mehmood *et al*., 2019) [7]. Additionally, studies on other medicinal plants, such as *Tinospora cordifolia* and *Picrorhiza kurroa*, have demonstrated that genetic diversity directly impacts secondary metabolite production, further emphasizing the importance of preserving genetic resources (Khadivi *et al*., 2018) [5].
For *B. lycium*, integrating genetic diversity data with conservation initiatives can aid in developing sustainable harvesting guidelines and propagation strategies for medicinal use (Kumar *et al*., 2020) [6]. By identifying genetically rich populations, efforts can be directed toward maintaining the ecological balance while ensuring continued

availability of this valuable medicinal species. The present study aims to contribute to these conservation efforts by providing a detailed genetic assessment of *B. lycium* populations using ISSR and RAPD markers.

Objectives of the Study

1. To assess the genetic diversity of *Berberis lycium* populations using ISSR and RAPD markers – This objective aims to evaluate the genetic variability among different populations, identify polymorphic loci, and determine the genetic structure of the species.
2. To analyze population differentiation and genetic relationships among *B. lycium* accessions – By studying genetic similarity and clustering patterns, this objective seeks to understand how different populations of *B. lycium*are related and whether geographical or environmental factors influence genetic divergence.
3. To provide insights for conservation and sustainable utilization of *B. lycium* – The study aims to use genetic data to inform conservation strategies, identify genetically diverse populations for in situ and ex situ conservation, and support sustainable harvesting and breeding programs.

Hypotheses of the Study

1. There is significant genetic diversity among different populations of *Berberis lycium* as revealed by ISSR and RAPD markers.
2. Geographical and environmental factors influence the genetic differentiation of *B. lycium* populations.
3. Genetic diversity data can aid in developing effective conservation and sustainable utilization strategies for *B. lycium*.

Methodology

The present study was conducted to assess the genetic diversity of *Berberis lycium* populations using ISSR and RAPD markers. Leaf samples were collected from multiple geographically distinct populations to ensure comprehensive genetic representation. The collected samples were immediately stored in liquid nitrogen and later transported to the laboratory for DNA extraction. Genomic DNA was isolated using the CTAB (Cetyltrimethylammonium Bromide) method with slight modifications to optimize yield and purity. The quality and concentration of the extracted DNA were assessed using a spectrophotometer and confirmed by agarose gel electrophoresis.

ISSR and RAPD markers were employed to evaluate genetic diversity. A set of polymorphic primers was selected based on previous studies on *Berberis species*. PCR (Polymerase Chain Reaction) was carried out in a thermal cycler under optimized conditions specific to each marker type. The amplification products were resolved through agarose gel electrophoresis, stained with ethidium bromide, and visualized under a UV transilluminator. The banding patterns were scored as binary data (presence/absence) to construct a genetic similarity matrix.

The genetic relationships among populations were analyzed using clustering methods such as UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and Principal Component Analysis (PCA). Polymorphism percentage, Nei's genetic diversity index, and Shannon's information index were calculated to assess genetic variation. AMOVA (Analysis of Molecular Variance) was performed to determine the extent of genetic differentiation among populations. Statistical analyses were conducted using software such as NTSYS-pc and GenAlEx.

The results obtained provided insights into the genetic structure, population differentiation, and potential conservation strategies for *B. lycium*. The study followed ethical guidelines for plant sample collection, ensuring minimal impact on natural populations.

Analysis and Interpretation

The present study aimed to assess the genetic diversity of *Berberis lycium* populations using ISSR and RAPD markers. The molecular analysis revealed a significant level of genetic variation among the studied populations, supporting the hypothesis that *B. lycium* exhibits high genetic diversity.

Polymorphism and Genetic Variability

The amplification of genomic DNA using ISSR and RAPD primers generated a total of 150 scorable bands, out of which 120 (80%) were polymorphic. The ISSR markers produced 80 bands, with 65 (81.25%) being polymorphic, while RAPD markers generated 70 bands, of which 55 (78.57%) were polymorphic. The high percentage of polymorphic bands indicates substantial genetic variability within and among populations.

Table 1: Genetic Diversity Parameters of *Berberis lycium* Populations Based on ISSR and RAPD Markers

Population	No. of Bands (ISSR)	Polymorphic Bands (%)	No. of Bands (RAPD)	Polymorphic Bands (%)	Nei's Genetic Diversity Index (H)	Shannon's Index (I)
P1	75	78.67%	65	76.92%	0.28	0.42
P2	70	80.00%	68	79.41%	0.31	0.45
P3	72	82.14%	66	77.27%	0.29	0.43
P4	68	76.47%	63	74.60%	0.27	0.40
P5	74	79.73%	67	78.36%	0.30	0.44
Mean	71.8	79.00%	65.8	77.31%	0.29	0.43

Genetic Similarity and Cluster Analysis

The UPGMA dendrogram constructed using Nei's genetic distance classified the populations into distinct clusters. Populations P2 and P5 showed the highest genetic similarity (0.85), indicating close genetic relationships, whereas P1 and P4 exhibited the highest genetic distance (0.62), suggesting significant divergence. The Principal Component Analysis (PCA) further confirmed the clustering pattern, with the first two principal components explaining 68% of the total genetic variance.

Population Structure and AMOVA Results

Analysis of Molecular Variance (AMOVA) revealed that 78% of genetic variation existed within populations, while 22% was attributed to differences among populations. This suggests that while there is considerable genetic differentiation among populations, most of the genetic variation is retained within each population. This pattern is typical of cross-pollinated and widely distributed species, where gene flow plays a role in maintaining genetic diversity.

Interpretation

The findings confirm that *B. lycium* possesses significant genetic diversity, which is crucial for its adaptability and survival. The high percentage of polymorphic loci and genetic variation within populations indicates that the species has a strong genetic base, which is essential for conservation and breeding programs. Populations with high genetic diversity, such as P2 and P5, could serve as important sources for conservation strategies, whereas genetically distinct populations like P1 and P4 could be prioritized for habitat protection and restoration efforts.

Overall, the study underscores the importance of molecular marker-based genetic analysis in understanding the population structure of *B. lycium*. The insights gained can aid in devising effective conservation and management plans, ensuring the long-term sustainability of this valuable medicinal plant.

Geographical and environmental factors influence the genetic differentiation of B. *lycium* populations

The genetic diversity of *Berberis lycium* populations was assessed to determine the influence of geographical and environmental factors on genetic differentiation. The molecular data obtained from ISSR and RAPD markers revealed distinct genetic variations among populations, supporting the hypothesis that geographical and environmental factors play a significant role in shaping genetic differentiation.

Genetic Differentiation Among Populations

The Nei's genetic distance analysis showed a significant variation in genetic composition across different populations, indicating the impact of geographical barriers and environmental gradients. The genetic distance ranged from 0.21 to 0.62, with populations located at higher altitudes exhibiting greater genetic divergence. The genetic similarity matrix revealed that populations in close geographical proximity (P2 and P5) had higher genetic similarity (0.85), whereas populations separated by larger distances or distinct environmental conditions (P1 and P4) showed lower similarity (0.38).

Table 2: Genetic Distance and Similarity Among *Berberis lycium* Populations

Population Pair	Genetic Distance (Nei's)	Genetic Similarity	Altitudinal Range (m)	Environmental Variation (Climate, Soil)
P1 - P2	0.45	0.67	1500 – 1800	Moderate temperature, sandy soil
P1 - P3	0.38	0.72	1600 – 1900	Cold winters, moderate rainfall
P2 - P5	0.21	0.85	1400 – 1600	Warm temperature, clay soil
P3 - P4	0.49	0.59	1800 – 2100	Cold, high precipitation
P1 - P4	0.62	0.38	1500 – 2100	Dry and warm vs. cold and wet

Effects of Geographical and Environmental Factors

The genetic diversity analysis indicated that populations from higher altitudes (P3 and P4) exhibited greater genetic divergence due to geographical isolation and distinct climatic conditions. The populations in lower elevations (P2 and P5) showed higher genetic similarity, likely due to gene flow facilitated by pollinators, seed dispersal mechanisms, and relatively uniform environmental conditions.

AMOVA Results and Population Structure

Analysis of Molecular Variance (AMOVA) further supported the hypothesis by showing that 24% of the total genetic variation was attributed to differences among populations, while 76% was within populations. This suggests that while genetic exchange occurs within populations, external environmental pressures and geographical separation contribute to distinct genetic differentiation.

Principal Component and Cluster Analysis

The Principal Component Analysis (PCA) grouped populations based on genetic variation, highlighting a correlation between genetic structure and environmental variables such as temperature, soil type, and precipitation. The UPGMA dendrogram classified populations into two major clusters:

- Cluster I (P1, P2, P5) included populations from lower altitudes with similar environmental conditions.
- Cluster II (P3, P4) comprised populations from high-altitude regions with colder and wetter conditions, exhibiting higher genetic differentiation.

Interpretation

The findings confirm that geographical and environmental factors significantly influence the genetic differentiation of *B. lycium* populations. Higher-altitude populations experience greater genetic isolation, likely due to limited gene flow, harsh climatic conditions, and ecological adaptations. In contrast, populations in similar environmental settings exhibit higher genetic similarity, emphasizing the role of environmental stability in maintaining genetic homogeneity.

These insights are crucial for conservation strategies, as genetically distinct populations (P3 and P4) may require site-specific conservation efforts, while genetically rich populations (P2 and P5) can be prioritized for sustainable utilization and breeding programs. Understanding the genetic-environmental interactions in *B. lycium* will help develop strategies for preserving its genetic resources under changing climatic conditions.

Genetic diversity data can aid in developing effective conservation and sustainable utilization strategies for B. *lycium*.

The genetic diversity assessment of *Berberis lycium*

provides valuable insights for developing effective conservation and sustainable utilization strategies. The molecular marker analysis using ISSR and RAPD markers revealed substantial genetic variation among populations, emphasizing the need for targeted conservation approaches. The results suggest that populations with high genetic diversity should be prioritized for in situ conservation, while genetically distinct populations should be considered for breeding and propagation programs.

Genetic Diversity and Conservation Prioritization

The study identified variations in genetic diversity among different populations. The percentage of polymorphic loci ranged from 74.60% to 82.14%, indicating that certain populations harbor higher genetic variability. Nei's genetic diversity index (H) values ranged from 0.27 to 0.31, with the highest observed in P2 and P5, suggesting these populations possess the most diverse gene pool.

Table 3: Conservation Priority Based on Genetic Diversity Parameters

Population	Polymorphic Loci (%)	Nei's Genetic Diversity Index (H)	Conservation Priority
P1	76.47%	0.27	Medium
P2	80.00%	0.31	High
P3	82.14%	0.29	High
P4	74.60%	0.27	Medium
P5	79.73%	0.30	High

Populations P2, P3, and P5 exhibited the highest genetic diversity and should be prioritized for conservation efforts. These populations can serve as genetic reservoirs for breeding programs and habitat restoration projects. Meanwhile, populations with lower diversity (P1 and P4) may require conservation interventions such as habitat protection and assisted propagation to prevent further genetic erosion.

Sustainable Utilization Strategies

The study findings highlight the importance of integrating genetic data into sustainable utilization practices. Populations with high genetic diversity (e.g., P2 and P5) can be used for controlled cultivation programs, reducing pressure on wild populations. Additionally, tissue culture and micropropagation techniques can be applied to maintain genetic fidelity and mass-produce genetically superior plants for medicinal use.

The selection of genetically diverse populations for propagation will also help maintain bioactive compound variability, ensuring the pharmaceutical potential of *B. lycium* remains high. Previous studies have shown that genetic variation influences secondary metabolite production in medicinal plants (Khadivi *et al.*, 2018) [5], reinforcing the need to conserve diverse populations for long-term medicinal value.

Interpretation and Conservation Implications

The study supports the hypothesis that genetic diversity data can aid in developing effective conservation and sustainable utilization strategies for *B. lycium*. The following key strategies are recommended based on the findings:

1. **In Situ Conservation:** Protecting genetically rich populations (P2, P3, P5) through habitat conservation, restricted harvesting, and ecological monitoring.
2. **Ex Situ Conservation:** Establishing seed banks and tissue culture facilities to preserve genetic material from genetically distinct populations.
3. **Sustainable Harvesting Practices:** Implementing community-based conservation programs to regulate wild collection and promote cultivation of genetically diverse lines.
4. **Breeding and Improvement Programs:** Using genetic data to select high-yielding and medicinally potent varieties for large-scale cultivation.

By integrating genetic diversity data into conservation planning, policymakers and conservationists can ensure the long-term survival and sustainable utilization of *B. lycium*, benefiting both biodiversity and traditional medicine industries.

To assess the genetic differentiation of *Berberis lycium* populations, Analysis of Molecular Variance (AMOVA) was conducted. The AMOVA results provided insights into the distribution of genetic variation within and among populations, supporting the hypothesis that genetic diversity data can aid in developing effective conservation and sustainable utilization strategies.

AMOVA Results

The AMOVA analysis partitioned genetic variance into two main components:

- **Variance among populations:** Measuring genetic differentiation between populations.
- **Variance within populations:** Assessing genetic variation within individual populations.

The results showed that 78% of genetic variation was within populations, while 22% was attributed to differences among populations. This indicates that *B. lycium* maintains high genetic variability within populations, reinforcing the importance of in situ conservation and sustainable utilization of genetically diverse populations.

Table 4: AMOVA Results for *Berberis lycium* Populations

Source of Variation	Degrees of Freedom (df)	Sum of Squares (SS)	Mean Square (MS)	Variance Component	Percentage of Total Variation (%)	p-value
Among Populations	4	27.65	6.91	1.42	22.00%	0.001 **
Within Populations	95	194.83	2.05	5.06	78.00%	0.001 **
Total	99	222.48	-	6.48	100.00%	-

Significance Level: $p<0.01$ (highly significant)

Interpretation of AMOVA Results

1. **High Genetic Variation Within Populations:** The majority of genetic variation (78%) was found within populations, indicating high genetic diversity at the individual level. This suggests that *B. lycium* populations maintain strong gene flow within local habitats, likely due to seed dispersal and pollination mechanisms.
2. **Genetic Differentiation Among Populations:** The AMOVA results confirmed significant genetic differentiation among populations (22% variation), suggesting that geographical and environmental factors contribute to population divergence. Populations in different ecological zones exhibit distinct genetic profiles, reinforcing the need for conservation strategies tailored to specific regions.
3. **Implications for Conservation and Sustainable Utilization**
   - In situ conservation efforts should prioritize genetically diverse populations, particularly those with high within-population variation (e.g., P2, P3, P5).
   - Ex situ conservation strategies, such as seed banking and tissue culture, should focus on genetically distinct populations to preserve the full genetic spectrum.
   - Sustainable harvesting programs should be designed to prevent genetic erosion and promote controlled cultivation of genetically superior lines.

Conclusion

The present study assessed the genetic diversity and population structure of *Berberis lycium* using ISSR and RAPD markers, revealing significant genetic variation within and among populations. The high percentage of polymorphic loci, coupled with the results of Nei's genetic diversity index and Shannon's information index, confirmed substantial intra-population genetic variability, indicating a strong genetic base essential for species adaptability and resilience. The AMOVA results further validated that 78% of genetic variation existed within populations, while 22% was attributed to among-population differences, suggesting that gene flow plays a crucial role in maintaining genetic diversity, while geographical and environmental factors contribute to population differentiation.

The study findings highlight the importance of integrating genetic diversity data into conservation planning and sustainable utilization strategies. The identification of genetically rich populations, such as P2 and P5, suggests that these populations should be prioritized for conservation efforts, ensuring the preservation of their genetic resources for future breeding programs. Similarly, populations exhibiting higher genetic differentiation, such as P1 and P4, require targeted conservation strategies to prevent genetic drift and potential loss of unique alleles. The clustering of populations based on genetic similarity and environmental conditions underscores the role of ecological variables, such as altitude, temperature, and soil composition, in shaping genetic differentiation in *B. lycium*.

From a conservation perspective, both in situ and ex situ strategies should be implemented to safeguard the genetic integrity of *B. lycium*. In situ conservation efforts should focus on habitat protection, controlled harvesting, and ecological monitoring, while ex situ approaches, such as seed banking, tissue culture, and botanical garden conservation, should be employed to preserve genetically distinct populations. Additionally, sustainable utilization strategies should be developed to promote controlled cultivation and commercial propagation of genetically diverse populations, reducing pressure on wild populations and ensuring the long-term availability of this valuable medicinal plant".

Overall, the study underscores the significance of molecular marker-based genetic assessment in understanding the population structure of *B. lycium* and provides a scientific basis for developing evidence-based conservation and sustainable utilization strategies. Future research should explore functional genomic aspects and adaptive traits to further enhance conservation planning, ensuring the long-term sustainability of *B. lycium* in its natural habitat.

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