Genetic Diversity of the Endangered Plant Tangut Rhubarb (Rheum tanguticum) on the Qinghai-Tibet Plateau (Postprint)

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Abstract

Rheum tanguticum is a traditional Chinese and Tibetan medicinal herb. In recent years, due to severe habitat destruction, it has become endangered and has been included in the list of endangered plant species. To explore the causes of endangerment of Rheum tanguticum and protect its wild resources, this study collected samples from 87 individuals across 9 populations of Rheum tanguticum, and conducted a genetic diversity analysis based on the chloroplast trnS-G gene sequence of this species. The results demonstrated that Rheum tanguticum possesses a relatively high level of genetic diversity (Ht=0.694), with 95.97% of genetic differentiation occurring among populations (GST=0.960) and 4.03% occurring within populations (Hs=0.028). AMOVA analysis also indicated low gene flow among populations (Nm=0.01) and high genetic differentiation (FST=0.9631). The relatively high genetic diversity of Rheum tanguticum may be associated with the species' long evolutionary history and life history characteristics, while the high genetic differentiation among populations may be related to the unique geographical environment of alpine regions and human activities. Based on these findings, we recommend implementing in-situ conservation for all wild populations of Rheum tanguticum, while concurrently collecting germplasm resources for ex-situ propagation to preserve the species' genetic diversity and maintain its evolutionary potential.

Full Text

Preamble

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Genetic Diversity of an Endangered Species, *Rheum tanguticum* (Polygonaceae), on the Qinghai-Tibetan Plateau

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Abstract

Rheum tanguticum is an endangered traditional Chinese and Tibetan medicinal plant that has been severely impacted by habitat destruction in recent years. To explore the causes of its endangerment and protect its wild resources, this study collected 87 individuals from nine populations of *R. tanguticum* and investigated their genetic diversity using the chloroplast *trnS-G* region sequence. The results revealed high genetic diversity within the species (total diversity Ht = 0.694), with significant genetic differentiation among populations (GST = 0.960). Approximately 95.97% of the genetic variation occurred among populations, while only 4.03% was attributed to within-population diversity. Analysis of Molecular Variance (AMOVA) confirmed this pattern (FST = 0.9631), and the gene flow estimate was extremely low (Nm = 0.01), indicating limited gene exchange between populations.

The high genetic diversity of *R. tanguticum* may be associated with its long evolutionary history and life history traits, while the strong genetic differentiation among populations likely reflects the unique alpine geographical environment and human activities in the region. Based on these findings, we recommend in-situ conservation of all wild populations, particularly those with high genetic diversity located far from human disturbance. Additionally, we suggest collecting and preserving seeds from genetically diverse populations for ex-situ conservation and propagation to maintain the species' evolutionary potential.

Keywords: *Rheum tanguticum*; *trnS-G* region; genetic diversity; gene flow

Introduction

The Qinghai-Tibetan Plateau is the world' s highest and largest plateau, representing one of the planet' s ecological hotspots with rich species diversity. Over 12,000 plant species grow in this region and its adjacent areas. Due to extreme environmental conditions such as high altitude and aridity, the ecosystem is

highly sensitive to environmental changes and human activities, with numerous plant species listed as endangered.

Rheum tanguticum Maxim. ex Balf. (Polygonaceae) is a perennial herb distributed primarily on the Qinghai-Tibetan Plateau. Valued for its anti-inflammatory properties and high quality in traditional Chinese and Tibetan medicine, the species has been over-exploited in recent years due to economic incentives, leading to severe population declines. Field surveys indicate that wild resources have become extremely scarce, making conservation of remaining populations urgent.

Genetic diversity studies are crucial for endangered species conservation, yet research on *R. tanguticum* has been limited. Previous investigations used SSR, ISSR, and other molecular markers, but no studies have employed the chloroplast *trnS-G* region, which offers relatively rapid evolutionary rates and high genetic variation suitable for intraspecific genetic diversity and gene flow analyses. This study addresses three key questions: (1) What is the genetic diversity level of *R. tanguticum*? (2) What is the genetic structure among populations? (3) What conservation strategies can be proposed based on these findings?

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1. Materials and Methods

1.1 Experimental Materials

We collected 87 *R. tanguticum* individuals from nine wild populations across the species' current distribution range on the Qinghai-Tibetan Plateau. Fresh leaves were silica-dried and stored for DNA extraction. Genomic DNA was isolated using a modified CTAB method. The chloroplast *trnS-G* intergenic region was amplified using primers designed by Hamilton: 'S' (5 -GCCGCTTTAGTCCACTCAGC-3) and 'G' (5 -GAACGAATCACACTTTACCAC-3).

PCR reactions (25 L total volume) contained 40 ng DNA, 17.5 L water, 2.5 L 10×Taq buffer, 0.5 L 10 mmol/L dNTPs, 5 mol/L primers (1.25 L each), and 1 U Taq DNA polymerase. Amplification conditions were: 94°C for 4.0 min; 35 cycles of 94°C for 1.0 min, 51°C for 1 min, 72°C for 1.75 min; and a final extension at 72°C for 8 min. PCR products were detected on 1% agarose gels, purified using a purification kit (Shanghai Sangon), and sequenced by Beijing Liuhe Huada Gene Technology Company. All sequences were submitted to GenBank.

1.2 Data Analysis

Sequences were aligned using Clustal X software with manual correction. DnaSP 5.1 was used to calculate haplotype diversity (Hd) and nucleotide diversity ( ), treating indels and base substitutions equally. PERMUT software calculated

within-population genetic diversity (Hs), total genetic diversity (Ht), and the genetic differentiation coefficient (GST). Gene flow (Nm) was estimated from GST values.

Arlequin 3.1 performed Analysis of Molecular Variance (AMOVA) with 1,000 permutations for significance testing. TFPGA software conducted Mantel tests (1,000 randomizations) to examine correlations between genetic and geographic distance matrices. NETWORK 4.6.1 constructed intraspecific haplotype networks using median-joining methods and coalescent theory to infer haplotype relationships. MEGA 6.0 generated Neighbor-Joining (NJ) trees based on genetic distances.

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2. Results

2.1 Sequence Variation and Haplotype Distribution

The *trnS-G* sequences from 87 samples ranged from 944 to 954 bp, with an aligned length of 965 bp. We detected 23 nucleotide variable sites, including 13 substitutions and 10 indels. Based on these mutations, four haplotypes (H1-H4) were identified. Haplotype H1 was the most widespread and abundant, present in five populations (Pop1, Pop2, Pop5, Pop7, Pop9) and accounting for 55.17% of all samples. Haplotype H4 occurred in two populations, while Pop3 and Pop8 each contained unique haplotypes. provides detailed sampling location information, and shows haplotype variation sites. presents haplotype distribution across populations.

The haplotype network revealed a star-like genetic structure ([Figure 2: see original paper]). Haplotypes H1, H2, and H4 occupied terminal positions, representing recent haplotypes, while H3 and three missing haplotypes were central. Haplotype H3, having the highest frequency, was inferred as the ancestral haplotype.

2.2 Genetic Diversity Analysis

At the species level, nucleotide diversity ( ) was 0.6218 and haplotype diversity (Hd) was 0.0049. Among the nine populations, Pop3 showed the highest haplotype and nucleotide diversity (Hd = 0.0026, = 0.0026), while three populations with only one haplotype had diversity values of 0. Within-population genetic diversity (Hs) averaged 0.028, indicating low within-population variation.

2.3 Genetic Structure Analysis

PERMUT analysis revealed strong genetic differentiation (GST = 0.960), with 95.97% of genetic variation occurring among populations and only 4.03% within populations. AMOVA confirmed this pattern (FST = 0.9631). The gene flow

estimate was extremely low ($Nm = 0.01$), indicating limited gene exchange between populations.

The NJ dendrogram based on genetic distances clustered Pop3 separately, while Pop7, Pop9, Pop5, and Pop1 formed a larger cluster ([Figure 3: see original paper]). Populations with close geographic proximity did not cluster together, suggesting no direct relationship between geographic and genetic distances, which was confirmed by the Mantel test ($r = 0.2461$, $P = 0.2060$).

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3. Discussion

3.1 Genetic Diversity of *Rheum tanguticum*

While reduced genetic diversity is often considered a primary cause of species endangerment, some endangered species maintain high genetic diversity, such as *Corydalis tomentella* (Papaveraceae) and *Glehnia littoralis* (Apiaceae). Our study revealed high genetic diversity in *R. tanguticum* based on chloroplast *trnS-G* sequences, consistent with previous SSR ($H = 0.515$), ISSR ($H = 0.2689$), and other marker studies.

This high diversity likely reflects the species' long evolutionary history. *Rheum* species diverged approximately 6.8–7.0 million years ago, and *R. tanguticum* has undergone extensive evolution, accumulating a rich gene pool. As a perennial herb with a complex outcrossing breeding system, the species possesses strong gene dispersal capabilities that maintain high genetic diversity.

3.2 Genetic Structure of *Rheum tanguticum* Populations

The species exhibits strong genetic differentiation among populations. Buso et al. consider $GST > 0.25$ as indicating extremely strong differentiation; our GST value of 0.960 far exceeds this threshold. AMOVA and PERMUT analyses consistently show that genetic variation primarily occurs among populations (95.97%), with minimal within-population diversity (4.03%).

This structure likely stems from the unique alpine environment. The plateau' s high mountains and deep valleys may impede pollen and seed dispersal, reducing gene flow between populations ($Nm = 0.01$). Over-harvesting may have eliminated some populations, disrupting continuous gene exchange and intensifying differentiation. The haplotype network supports this, showing missing ancestral haplotypes and a star-shaped pattern indicating recent expansion from glacial refugia.

Clustering analysis revealed that geographically close populations did not group together, and the Mantel test showed no significant correlation between geographic and genetic distances ($r = 0.2461$, $P = 0.2060$). This suggests that current populations may have originated from different glacial refugia, with genetic drift during post-glacial expansion causing haplotype loss. For example,

Tibetan and Qinghai populations clustered in separate branches, supporting a history of glacial retreat and interglacial expansion.

3.3 Conservation of *Rheum tanguticum* Genetic Diversity

Genetic variation is fundamental for species evolution, making its conservation essential for endangered species. Hamrick and Godt suggest that if a population' s genetic diversity represents a substantial proportion of total species diversity, it should be protected separately; otherwise, all populations require protection.

Our results show that while Pop3 has the highest diversity ($H\mathrm{d} = 0.0026$), it represents only 53.06% of total genetic diversity, not meeting the threshold for separate protection. Given that most genetic diversity exists among populations and gene flow is severely limited, we recommend:

1. **In-situ conservation** of all wild populations, including those with lower diversity but unique alleles, to maintain population numbers and ensure inter-population gene exchange.
2. **Ex-situ conservation** through seed collection and germplasm resource banks, particularly if in-situ measures prove insufficient.
3. **Propagation programs** to increase population numbers and expand distribution range.

These strategies will preserve evolutionary potential and prevent extinction due to genetic erosion.

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4. Conclusion

This study analyzed genetic structure in nine wild *R. tanguticum* populations using chloroplast *trnS-G* sequences. The species maintains high genetic diversity, but with strong differentiation among populations and low gene flow, likely resulting from alpine habitat isolation and human disturbance. To conserve this genetic diversity, we recommend comprehensive in-situ protection of all wild populations combined with ex-situ seed preservation and propagation programs. These measures will help increase population numbers and expand the species' distribution.

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Note: Figure translations are in progress. See original paper for figures.