Received: 20 June 2025 | Revised: 7 December 2025 | Accepted: 11 December 2025

DOI: 10.1111/1442-1984.70047

ORIGINAL ARTICLE

PLANT SPECIES BIOLOGY WILEY

Effects of environmental factors on genetic diversity in the endangered *Notopterygium incisum* from the southeastern Qinghai–Tibetan Plateau

Jinqiu Liao[1] | **Ao Luo**[1] | **Rui Zeng**[1] | **Xianhong Feng**[2] | **Yuanyuan Jiang**[3] | **Xuexue Deng**[3] | **Li Zhang**[3] | **Ruiwu Yang**[1]

[1]College of Life Science, Sichuan Agricultural University, Ya'an, China

[2]Cash Crops Research Laboratory, Ganzi Tibetan Autonomous Prefecture Institute of Agricultural Sciences, Kangding, China

[3]College of Science, Sichuan Agricultural University, Ya'an, China

Correspondence
Li Zhang, College of Science, Sichuan Agricultural University, Ya'an 625014, China.
Email: zhangli@sicau.edu.cn

Ruiwu Yang, College of Life Science, Sichuan Agricultural University, Ya'an 625014, China.
Email: yrwu@sicau.edu.cn

Abstract

Notopterygium incisum is an endangered medicinal plant species endemic to China, primarily distributed in the eastern peripheral regions of the Qinghai–Tibetan Plateau. As a widely utilized medicinal plant, *N. incisum* faces increasing threats from environmental changes and anthropogenic disturbances, which are altering its biological habitats and elevating its risk of extinction. These challenges pose significant obstacles to the species' breeding and conservation. To inform more effective breeding strategies and conservation measures, this study investigated the genetic diversity, population differentiation, and evolutionary history of *N. incisum*, as well as the environmental influences on 343 individuals from 38 natural populations, using 15 ISSR markers. The results reveal a moderate level of genetic diversity across the sampled populations. The 38 populations were grouped into two major clusters derived from a common ancestor. Genetic diversity was found to be correlated with altitude, latitude, and average annual temperature, and pop35 and pop27 exhibited the highest genetic diversity indices, representing valuable genetic germplasm for breeding programs. These populations may serve as excellent parental lines to improve breeding efficiency and success. Notably, populations located in Aba, Sichuan Province, at altitudes between 2500 and 3800 m and latitudes of 32–33°N, showed higher genetic diversity, suggesting stronger adaptive capacity in these environments. This finding provides a theoretical basis for selecting experimental sites for future breeding efforts. For conservation, in situ strategies are recommended for populations with high genetic diversity. Those under significant environmental threat may benefit from ex situ conservation, such as seed or rhizome collection, to maximize genetic representation.

Jinqiu Liao and Ao Luo contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2026 The Author(s). *Plant Species Biology* published by John Wiley & Sons Australia, Ltd on behalf of The Ecological Society of Japan.

KEYWORDS

environmental factors, genetic diversity, ISSR markers, *Notopterygium incisum*, plant breeding

1 | INTRODUCTION

Notopterygium incisum Ting ex H. T. Chang, a widely utilized medicinal plant, belongs to the family Apiaceae (She & Watson, 2005). It typically grows in alpine shrublands, subalpine thickets, and along alpine forest margins, at elevations ranging from 1700 to 4500 m (Yang et al., 2019). As a psychrophyte, *N. incisum* is distributed in the eastern peripheral regions of the northeastern Qinghai–Tibetan Plateau (QTP), encompassing parts of Sichuan, Qinghai, and Gansu provinces (Liu et al., 2019).

The herbal medicine derived from the rhizomes and roots of *N. incisum* is officially documented in the Chinese Pharmacopoeia (Chinese Pharmacopoeia Committee, 2020). It is commonly employed for the treatment of the common cold and inflammatory conditions, such as rheumatoid arthritis, and functions as a diaphoretic, antipyretic, and analgesic agent (Azietaku et al., 2017). As of 2017, a total of 694 pharmaceutical manufacturers in China utilized *N. incisum* rhizomes as raw materials for drug production (Jia et al., 2019). Growing demand in both domestic and international medicinal markets has led to a rapid increase in the market requirement for this plant. However, the current supply predominantly depends on the harvesting of wild resources, which has significantly contributed to its increasing threat of extinction. Over-exploitation and other human activities have resulted in habitat degradation and fragmentation for *N. incisum* (Jia et al., 2019). Consequently, *N. incisum* has been classified as an endangered species on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species and is recognized as a traditional Tibetan medicinal plant at risk (Wang & Xie, 2005).

The assessment of genetic polymorphisms in threatened species constitutes a prerequisite for germplasm conservation. In recent years, the use of molecular markers to evaluate genetic diversity in endangered plant populations has become a critical step in developing effective conservation strategies. Although several studies have investigated the genetic diversity of *N. incisum* (Tang et al., 2008; Yang et al., 2015), these were limited to only 3–4 populations or relied on a single nuclear gene. Therefore, it is necessary to expand the sample size and employ multiple molecular marker techniques to explore genetic diversity among and within *N. incisum* populations in greater detail. Moreover, the correlation between genetic diversity and environmental factors remains to be investigated. Based on such findings, effective conservation strategies can be formulated to ensure the sustainable utilization of *N. incisum* resources.

Genetic diversity determines the morphological differences, chemical composition, and content differences among different populations, plants, and environmental adaptations (Nevo et al., 1998; Semagn et al., 2000). Genetic diversity resulting from geographical variation is associated with environmental factors such as latitude, altitude, temperature, and precipitation (Li et al., 1996; Yang et al., 2019). Based on the analysis of Internal Transcribed Spacer sequence (ITS) sequences from 23 different populations of *N. incisum* germplasm resources, higher genetic diversity in *N. incisum* was generally associated with environments characterized by lower latitude, greater precipitation, higher altitude, and more stable climatic conditions (Yang et al., 2019). In *Saussurea obvallata*, both inter- and intra-population genetic diversity were relatively low, exhibited a significant correlation with geographical distance, and revealed a pronounced decreasing trend from east to west. Furthermore, metabolite content varied substantially among populations, a variation primarily associated with average annual temperature and temperature fluctuations (Zhang et al., 2025). Molecular marker analyses, employing inter-simple sequence repeat (ISSR) and single nucleotide polymorphisms (SNPs), on the genetic structure of *Foeniculum vulgare* Mill. germplasm identified key loci associated with agronomically important traits, including seed yield and disease resistance. These findings pave the way for developing improved cultivars through an integrated approach that combines classical breeding with modern biotechnology (Giachino & Boztaş, 2025). The correlation between environmental factors and genetic diversity plays a crucial role in understanding the formation mechanisms of authentic medicinal materials, the conservation of endangered medicinal plants, and the breeding of new varieties. Although several studies have investigated the genetic diversity of *N. incisum* (Yang et al., 2015), there remains a need to incorporate more molecular marker methods and examine a wider range of populations. Such approaches are essential to elucidate

the relationship between genetic diversity and environmental adaptability in *N. incisum*. A deeper understanding of this relationship will help identify the key environmental factors influencing the species and provide a foundation for breeding new varieties, as well as for analyzing the mechanisms underlying the formation of its active compounds.

In this study, ISSR markers were employed to analyze the genetic diversity, genetic differentiation, and evolutionary history of 343 individuals from 38 populations of *N. incisum* across its natural distribution in the southeastern Qinghai–Tibetan Plateau (QTP), encompassing regions of Sichuan, Gansu, and Qinghai provinces in China. Mantel tests and redundancy analysis (RDA) were applied to examine the correlations between genetic variation and climatic variables, geographical factors, and altitudinal gradients. These analyses aimed to elucidate the spatial genetic patterns of *N. incisum* within the southeastern QTP. The results will provide insights into the influence of environmental factors on the species' genetic diversity. Furthermore, understanding the genetic diversity and environmental adaptability of *N. incisum* will facilitate the selection of parental materials and suitable breeding environments, thereby accelerating the breeding process. Based on these findings, strategies for the protection of wild *N. incisum* resources in the southeastern QTP will be proposed.

2 | MATERIALS AND METHODS

2.1 | Plant material

Notopterygium incisum is an endangered plant in China. Its main distribution area is on the Northwest Sichuan Plateau, which is located on the eastern edge of the Qinghai–Tibetan Plateau (QTP). The study collected 343 leaf samples from 38 *N. incisum* populations in Ganzi and Aba prefectures of Sichuan, as well as the borders between Qinghai, Gansu, and Sichuan during 2020–2021 (Table 1). At sites with higher plant density, a greater number of samples (e.g., 10 individuals) were collected to adequately capture the population's genetic diversity. In contrast, at locations with lower density, sampling effort was reduced (e.g., six individuals) to limit disturbance to local plant communities and conserve resources. Within each site, samples were collected along a zigzag transect, maintaining a minimum interval of 50 m between consecutive individuals to ensure spatial independence and representation. All the samples were stored at −80°C until DNA extraction.

The taxonomic identification of all collected *N. incisum* specimens was performed by Professor Ruiwu Yang at Sichuan Agricultural University. The detailed geographic location data for the 38 sampled populations are provided in Table 1 and illustrated in Figure 1.

2.2 | DNA extraction

Genomic DNA was extracted from approximately 100 mg of leaf tissue using the LABGENETM Plant DNA Isolation Kit (LABGENE, Cat: LB1211) according to the manufacturer's protocol. DNA concentration and purity were quantified using a NanoDrop 1000 spectrophotometer, and integrity was verified by electrophoresis on a 1% agarose gel stained with TS-GelRed. DNA samples meeting the following quality criteria—concentration ≥50 ng/μL, A_{260}/A_{280} ratio between 1.8 and 2.0, and total yield ≥1 μg—were stored at −80°C for subsequent molecular analyses.

2.3 | ISSR-PCR amplification

DNA extracted from individual samples across 10 populations was amplified using 37 ISSR primers from the University of British Columbia (UBC) Primer Set No. 9 (Table S1). PCR reactions were carried out using the TIANGEN 2 × Taq PCR MasterMix II (TIANGEN, Cat: KT 2011201X) according to the manufacturer's instructions. Amplification products were separated by electrophoresis on 1.5% agarose gels stained with TS-GelRed in 1 × TAE buffer and visualized under UV light using a BIO-RAD gel documentation system (Image Lab Software v6.1). Primers producing clear, reproducible, and polymorphic band patterns were selected for subsequent amplification of all 343 individuals.

2.4 | Genetic data analysis

Amplified bands were scored in a binary matrix as present (1) or absent (0). The number of total bands, polymorphic bands, and the percentage of polymorphic loci (PPL) were calculated. Genetic diversity was assessed using the following indices: observed number of alleles (*Na*), effective number of alleles (*Ne*), *Nei*'s genetic distance (*H*), and Shannon's information index (*I*). Total gene diversity (*Ht*), within-population diversity (*Hs*), genetic differentiation coefficient (*Gst*), and gene flow (*Nm*) were also estimated. All calculations were performed in PopGene version 1.32 based on a diploid dominant model, with significance tested over 1000 permutations (Yeh et al., 1999).

A pairwise genetic distance matrix (Table S2) was constructed using PopGene and used to generate a dendrogram via the unweighted pair-group method with

TABLE 1 Sampling details and sample sizes of the 38 *Notopterygium incisum* populations.

No.	ID	Location	Individuals no.	Longitude (°)	Latitude (°)	Altitude (m)
1	pop1	Xiaojin, Aba, Sichuan	10	102.4036	30.7880	4469
2	pop2	Xinlong, Ganzi, Sichuan	10	100.6994	30.5754	4274
3	pop3	Xiaojin, Aba, Sichuan	7	102.8908	30.9455	4309
4	pop4	Dege, Ganzi, Sichuan	10	99.0475	32.4109	3722
5	pop5	Seda, Ganzi, Sichuan	8	100.8894	31.8249	4184
6	pop6	Maerkang, Aba, Sichuna	6	102.2721	31.7892	3322
7	pop7	Xiaojin, Aba, Sichuan	9	102.4989	31.4895	3179
8	pop8	Yajiang, Ganzi, Sichuan	10	101.1438	30.0793	3266
9	pop9	Danba, Ganzi, Sichuan	10	101.5174	31.2325	3689
10	pop10	Jinchuan, Aba, Sichuan	7	103.8864	31.7519	3200
11	pop11	Maoxian, Aba, Sichuna	10	103.5070	31.8338	3558
12	pop12	Daofu, Ganzi, Sichuan	8	101.6252	30.5335	3749
13	pop13	Litang, Ganzi, Sichuan	10	100.3049	30.3309	3894
14	pop14	Daofu, Ganzi, Sichuan	10	101.4907	30.4889	3468
15	pop15	Xiaojin, Aba, Sichuan	9	102.4556	31.2478	2988
16	pop16	Luding, Ganzi, Sichuan	10	102.0339	29.8823	3939
17	pop17	Songpan, Aba, Sichuan	10	103.6097	32.6840	3126
18	pop18	Minxian, Singxi, Gansu	6	104.0968	34.9383	3027
19	pop19	Danba, Aba, Sichuan	9	101.8701	30.9527	2279
20	pop20	Ruoergai, Aba, Sichuan	10	103.3766	33.4730	3379
21	pop21	Maoxian, Aba, Sichuna	6	103.5732	32.1679	3272
22	pop22	Heihui, Aba, Sichuan	10	102.8174	32.1075	2772
23	pop23	Songpan, Aba, Sichuan	9	103.7485	32.4831	3330
24	pop24	Jiuzhaigou, Aba, Sichuan	9	104.1598	33.0880	1945
25	pop25	Zhouqu, Gannan, Gansu	10	104.3445	33.4343	2634
26	pop26	Maqu, Gannan, Gansu	10	101.1289	34.2479	3468
27	pop27	Danba, Ganzi, Sichuan	10	101.1855	31.2904	3740
28	pop28	Seda, Ganzi, Sichuan	9	100.7364	31.8772	3391
29	pop29	Jiuzhi, Guoluo, Qinghai	10	101.0392	33.8035	3633
30	pop30	Yajiang, Ganzi, Sichuan	10	100.5266	33.7814	4201
31	pop31	Banma, Guoluo, Qinghai	7	100.8753	32.7186	3708
32	pop32	Hongyuan, Aba, Sichuan	9	102.9800	33.0153	3519
33	pop33	Lixian, Aba, Sichuan	10	102.8108	31.6655	2962
34	pop34	Diebu, Gannan, Gansu	6	103.2158	34.0634	2477
35	pop35	Maerkang, Aba, Sichuna	10	102.1132	31.9246	2777
36	pop36	Aba, Aba, Sichuna	10	102.0644	32.7464	3443
37	pop37	Rangtang, Aba, Sichuan	9	101.0737	32.4221	3436
38	pop38	Jinchuan, Aba, Sichuan	10	101.5531	31.8050	2905

arithmetic averages (UPGMA) in MEGA version 6 (Tamura et al., 2013).

Genetic distances between populations were centralized, standardized, and normalized to z-scores prior to principal component analysis (PCA) to visualize population structuring.

Molecular variance was partitioned within and among populations through analysis of molecular

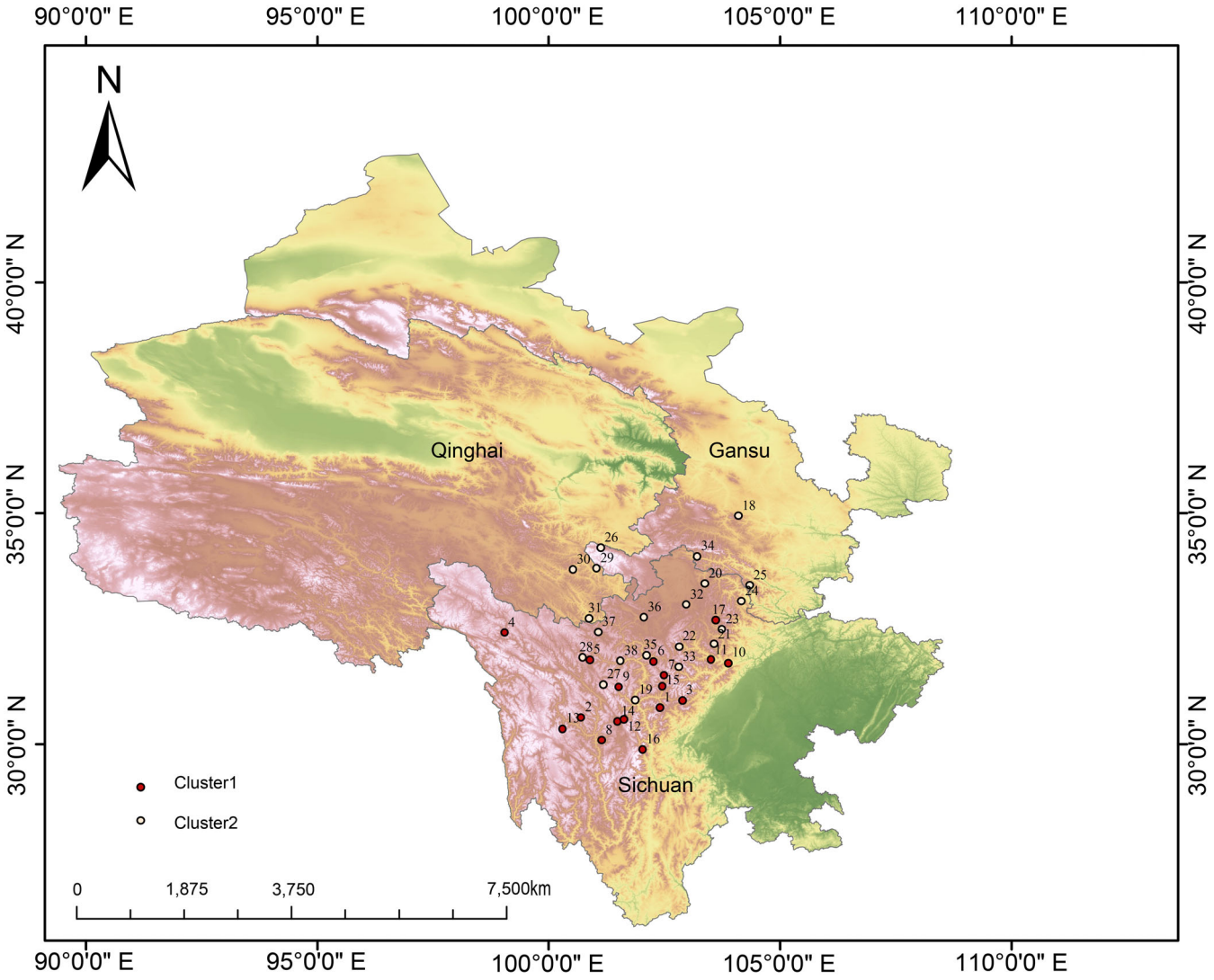


FIGURE 1 Sampling locations of 38 *Notopterygium incisum* populations in the southeastern Qinghai–Tibet Plateau, China.

variance (AMOVA) implemented in Arlequin version 3.5.2.2, with significance evaluated using 10 000 permutations (Excoffier & Lischer, 2010).

2.5 | Analysis of population evolution history

The evolutionary history was inferred with Do-It-Yourself Approximate Bayesian Computation (DIYABC) (Collin et al., 2021) using a generalized stepwise mutation model (GSM) to evaluate alternative evolutionary scenarios and estimate historical parameters. The 38 *N. incisum* populations were categorized into two distinct gene pools, designated N1 (populations 1–18) and N2 (populations 19–38). Three evolutionary scenarios were tested: (1) N1 and N2 diverged simultaneously from a common ancestral population (N1 + N2) at time t_a; (2) N1 was the ancestral group, with N2 diverging from it at time t_a; and (3) N2 was the ancestral group, with N1 diverging from it at time t_a. Following the simulation of 100 000 training datasets, the optimal scenario was identified using logistic regression based on the top 1% of simulated datasets closest to the observed data. Subsequently, historical parameters were estimated under the best-supported model. The prior distributions for all model parameters are provided in Table S3.

2.6 | Environmental data collection

Geographic distances between populations were calculated based on their latitude and longitude using GenAlEx 5.6 (Peakall & Smouse, 2012). Altitude data and monthly climate variables (precipitation, average temperature, maximum and minimum temperatures) for the period 2000–2021, all at a 1 km resolution, were obtained from WorldClim and the National Qinghai–Tibet Plateau

Scientific Data Center (data.tpdc.ac.cn/home), respectively. These datasets were processed in ArcMap 10.4 to extract values corresponding to each population's location. We then derived three key annual bioclimatic variables for the 22-year period: (1) average annual precipitation (Bio1), calculated as the total monthly precipitation averaged over 22 years; (2) average annual temperature (Bio2), calculated as the total monthly temperature averaged over 22 years; and (3) average annual temperature range (Bio3), calculated as the average of the monthly difference between maximum and minimum temperatures over 22 years.

2.7 | Correlation analysis between genetic diversity and environmental factors

Multiple analytical methods were employed to identify the geographical and climatic variables that may account for the variation in genetic diversity among different *N. incisum* populations. Pearson's correlation analysis was conducted to assess the relationships between genetic diversity parameters and geographical/climatic variables (Hollander et al., 2015). A significance threshold of $p < 0.05$ was applied, with an asterisk (*) indicating statistically significant correlations.

A Mantel test was performed to evaluate the correlations between genetic distance and geographical distance, as well as between genetic distance and geographical or climatic factors. Climate variables, together with longitude, latitude, and altitude data, were converted into a Euclidean distance matrix using SPSS 26, based on pairwise differences between populations. Using genetic distance data along with geographical and climatic variables from the 38 populations, the Mantel test was implemented in GenAlEx 5.6 to examine the presence of geographical or environmental isolation among populations (Peakall & Smouse, 2012).

Redundancy analyses (RDAs) were applied to investigate the linear relationships between genetic diversity parameters and multivariate environmental gradients. This analysis was carried out using the OmicStudio platform available at www.omicstudio.cn/tool (Ter Braak, 1988).

3 | RESULTS

3.1 | Genetic diversity

Initial screening revealed that, out of the 37 ISSR primers tested, 15 primers exhibited clear and reproducible polymorphic bands. These 15 primers were subsequently employed to amplify DNA from 343 *N. incisum* individuals (Table 2). A total of 203 loci were amplified, with the number of loci per primer ranging from 8 (UBC824) to 20 (UBC842). The amplified fragment sizes varied

TABLE 2 List of ISSR-PCR primers used in the study and their amplification profiles.

Primers	Sequence (5′–3′)	TB	PB	PPL	AS (bp)
UBC808	AGAGAGAGAGAGAGAGC	10	10	100	100–1500
UBC811	GAGAGAGAGAGAGAGAC	12	12	100	100–1500
UBC827	ACACACACACACACACG	12	12	100	100–1000
UBC834	CTCTCTCTCTCTCTCTG	11	11	100	100–1500
UBC835	AGAGAGAGAGAGAGAGYC	13	13	100	100–2000
UBC842	GAGAGAGAGAGAGAGAYG	20	20	100	100–2000
UBC841	GAGAGAGAGAGAGAGAYC	18	18	100	100–2000
UBC836	AGAGAGAGGAGAGAGYA	16	16	100	100–2000
UBC809	AGAGAAGAGAGAGAGG	14	14	100	100–2000
UBC823	TCTCTCTCTCTCTCTCC	12	12	100	100–2000
UBC824	TCTCTCTCTCTCTCTCG	8	8	100	100–2000
UBC840	GAGAGAGAGAGAGAGAYT	14	14	100	100–2000
UBC855	ACACACACACACACACYT	13	13	100	100–2000
UBC879	CTTCACTTCACTTCA	14	14	100	100–2000
UBC899	CATGGTGTTGGTCATTGTTCCA	16	16	100	100–2000
Average		13.5	13.5	100	
Total		203	203	100	

Abbreviations: AS (pb), amplicon size in base pairs; PB, polymorphic bands; PPL, percentage of polymorphic loci; TB, total bands.

TABLE 3 Genetic diversity parameters of the 38 *Notopterygium incisum* populations.

pop	*Na*	*Ne*	*H*	*I*	PPL (%)
pop1	1.6552	1.3637	0.2140	0.3238	65.52
pop2	1.6798	1.4106	0.2386	0.3565	67.98
pop3	1.5961	1.3597	0.2106	0.3152	59.61
pop4	1.6650	1.3589	0.2169	0.3305	66.50
pop5	1.5764	1.3376	0.1985	0.2988	57.64
pop6	1.6601	1.3736	0.2217	0.3352	66.01
pop7	1.7241	1.4149	0.2444	0.3684	72.41
pop8	1.6897	1.3679	0.2206	0.3366	68.97
pop9	1.6502	1.3839	0.2258	0.3393	65.02
pop10	1.6798	1.3851	0.2263	0.3413	67.98
pop11	1.6798	1.3919	0.2306	0.3472	67.98
pop12	1.7094	1.3705	0.2232	0.3417	70.94
pop13	1.6897	1.3871	0.2294	0.3468	68.97
pop14	1.7340	1.4010	0.2387	0.3628	73.40
pop15	1.6995	1.4359	0.2517	0.3741	69.95
pop16	1.7389	1.3882	0.2333	0.3563	73.89
pop17	1.6256	1.3694	0.2183	0.3283	62.56
pop18	1.6897	1.3780	0.2259	0.3425	68.97
pop19	1.6404	1.3435	0.2085	0.3184	64.04
pop20	1.7586	1.4105	0.2436	0.3701	75.86
pop21	1.7931	1.4044	0.2459	0.3777	79.31
pop22	1.7685	1.3963	0.2405	0.3687	76.85
pop23	1.7833	1.3758	0.2331	0.3615	78.33
pop24	1.7685	1.3929	0.2411	0.3700	76.85
pop25	1.7980	1.4342	0.2574	0.3906	79.80
pop26	1.7537	1.3919	0.2348	0.3589	75.37
pop27	1.8030	1.4442	0.2640	0.3996	80.30
pop28	1.7833	1.4308	0.2575	0.3908	78.33
pop29	1.7340	1.4309	0.2520	0.3786	73.40
pop30	1.7438	1.4099	0.2451	0.3716	74.38
pop31	1.7488	1.4247	0.2521	0.3805	74.88
pop32	1.6502	1.3887	0.2275	0.3412	65.02
pop33	1.7833	1.4378	0.2603	0.3941	78.33
pop34	1.7685	1.4392	0.2592	0.3911	76.85
pop35	1.7685	1.4848	0.2769	0.4109	76.85
pop36	1.7340	1.4204	0.2492	0.3757	73.40
pop37	1.7143	1.4464	0.2587	0.3850	71.43
pop38	1.7980	1.4657	0.2725	0.4096	79.80
Average	1.7168	1.4013	0.2381	0.3603	71.67

Note: *Na*, observed number of alleles; *Ne*, effective number of alleles; *H*, *Nei*'s genetic distance; *I*, Shannon's information index.
Abbreviation: PPL, percentage of polymorphic loci.

between 100 and 2000 bp, and all primers demonstrated 100% polymorphism.

Genetic diversity parameters were calculated from the amplification profiles using PopGene version 1.32 (Table 3). The mean number of alleles per locus (*Na*) ranged from 1.5764 (pop5) to 1.8030 (pop27), with an average of 1.7168. The effective number of alleles (*Ne*) averaged 1.4013, ranging from 1.3376 (pop5) to 1.4848 (pop35). The observed heterozygosity (*H*), Shannon's information index (*I*), and percentage of polymorphic loci (PPL) ranged from 0.1985 (pop5) to 0.2769 (pop35), 0.2988 (pop5) to 0.4109 (pop35), and 57.64% (pop5) to 80.30% (pop27), respectively. The corresponding mean values were 0.2381 for *H*, 0.3603 for *I*, and 71.67% for PPL (Table 3).

Populations pop7, pop20, pop21, pop25, pop27, pop28, pop29, pop30, pop31, pop33, pop34, pop35, pop36, and pop38 displayed relatively high genetic diversity. In contrast, lower genetic diversity was observed in populations pop1, pop3, pop4, pop5, pop6, pop8, pop9, pop10, pop11, pop12, pop13, pop17, pop18, and pop19. At the species level, the values for *H* (0.2381) and *I* (0.3603) indicate a moderate level of genetic variation in *N. incisum*.

3.2 | Genetic relationships

To assess the genetic relationships among the 38 *N. incisum* populations, an Unweighted Pair Group Method with Arithmetic Mean Dendrogram (UPGMA) was constructed based on *Nei*'s genetic distance (Figure 2). The clustering analysis revealed a clear division of the populations into two major groups: Cluster I, comprising populations pop1 to pop18, and Cluster II, comprising populations pop19 to pop38.

This bipartite grouping was further supported by a principal component analysis (PCA), with the first principal component (PCA1) effectively separating all populations into the same two clusters (Figure 3; Table S4). Geographically, Cluster I, characterized by lower genetic diversity, is predominantly distributed in the southern region (29–32° N) of the Northwestern Sichuan Plateau. In contrast, Cluster II, which exhibits higher genetic diversity, is mainly located in the northern region (32–35° N) of the same plateau.

The gene flow estimates (*Nm*) were 1.7346 among all populations and 18.0461 between the two clusters (Table 4). Since both values exceed 1, this indicates a high level of gene flow in *N. incisum*, sufficient to counteract genetic differentiation caused by genetic drift.

An analysis of molecular variance (AMOVA) was also performed to partition the genetic variance within and among populations (Table 5). The results showed that

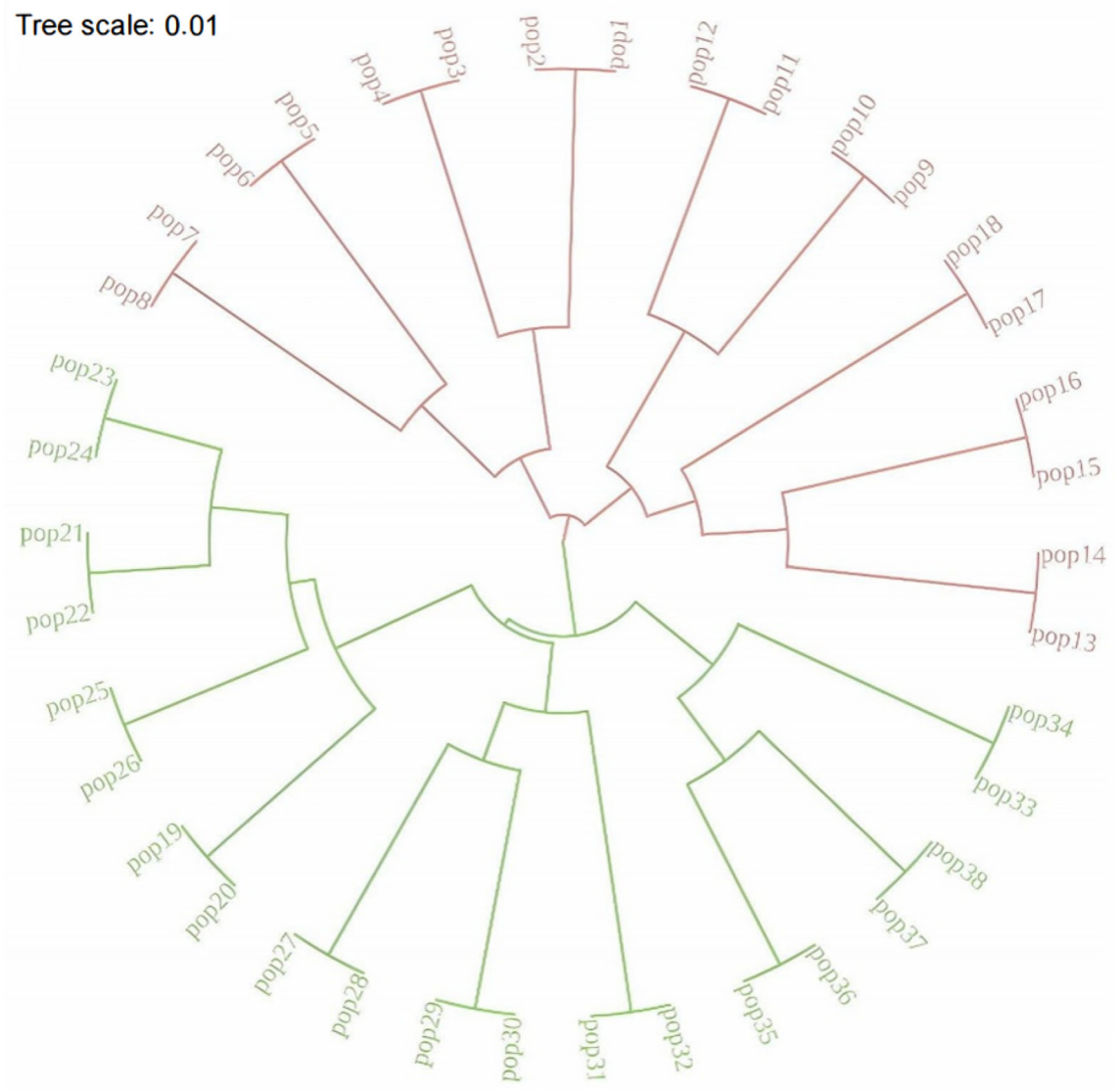


FIGURE 2 UPGMA dendrogram of 38 *Notopterygium incisum* populations based on *Nei*'s genetic distance (*H*). Population codes correspond to those listed in Table 1.

79.99% of the total genetic variation occurred within populations, while only 20.01% was attributed to variation among populations. This finding is consistent with the *Gst* value (27.64%) obtained from PopGene, further supporting the conclusion that genetic differentiation in *N. incisum* is predominantly within populations. Between the two clusters, only 2.77% of the genetic variation was observed, indicating that the level of differentiation has not yet reached a degree sufficient for subspecies formation.

3.3 | Analysis of population evolutionary history

A Do-It-Yourself Approximate Bayesian Computation (DIYABC) analysis identified Scenario 1 as the most probable evolutionary history, with the highest posterior probability (Table S5). This scenario posits that Cluster I and Cluster II diverged simultaneously from a common ancestral population at time t_a (Figure 4a). Under Scenario 1, the posterior distributions of parameters were estimated (Table 6). The effective population size for the ancestral population (N1) was 5473, while the derived population (N2) had an effective size of 4949.

Assuming a generation time of 3 years for *N. incisum*, based on its life cycle of 2–4 years (Wu & Raven, 2005), the divergence time between the two clusters was estimated to be approximately 14 928 years before present. This timeframe corresponds to the Last Glacial Maximum, suggesting that the divergence of *N. incisum* coincided with, and may have been driven by, Quaternary glacial cycles, potentially

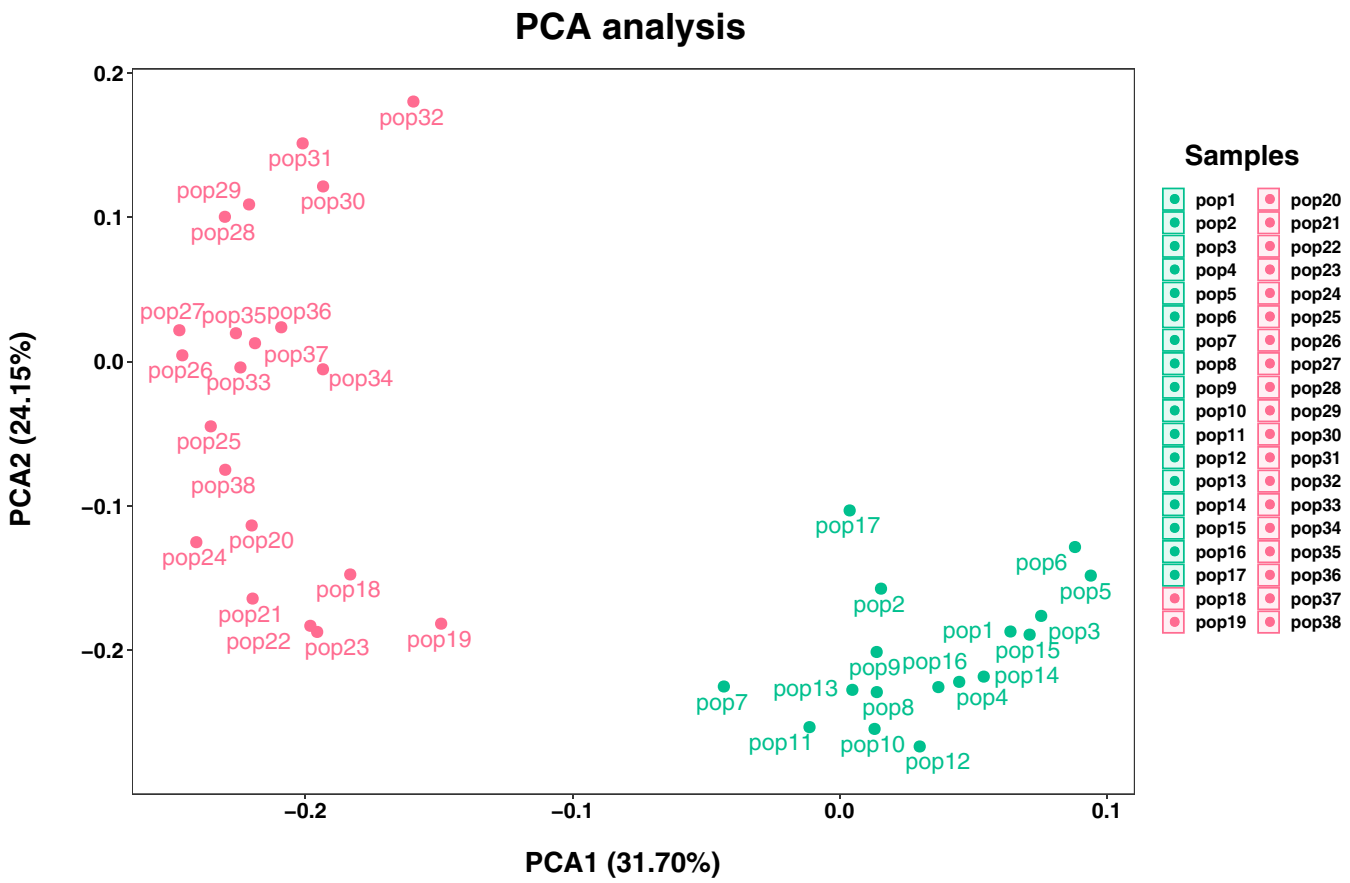


FIGURE 3 Principal component analysis (PCA) of 38 *Notopterygium incisum* populations. Population codes are provided in Table 1.

TABLE 4 Genetic differentiation indices (*Ht*, *Hs*, *Gst*, and *Nm*) of *Notopterygium incisum*.

Group	*Ht*	*Hs*	*Gst*	*Nm*
Among populations	0.3381	0.2400	0.2764	1.7346
Two clusters	0.3122	0.3038	0.0270	18.0461

Note: *Ht*, total gene diversity; *Hs*, within-population diversity; *Gst*, genetic differentiation coefficient; *Nm*, gene flow.

involving a short-term population expansion as an adaptation to the changing glacial environment.

3.4 | Correlation between genetic diversity and environmental factors

Correlation analyses were performed to assess the relationships between genetic diversity parameters (*Na*, *Ne*, *H*, *I*) and geographic distance, topographic factors, and climatic variables (Figures 5 and 6). No significant correlations were observed between any genetic diversity parameter and geographic distance (Figure 5), indicating that isolation-by-distance is not a primary factor shaping genetic variation in *N. incisum*. In contrast, several environmental factors showed significant associations with genetic diversity. Average annual temperature (Bio2) was positively correlated with all genetic diversity indices (*Na*, *Ne*, *H*, *I*; $p < 0.05$). Latitude also showed a significant positive correlation with Shannon's Information Index (*I*; $p < 0.05$), while altitude was negatively correlated with *Na*, *H*, and *I* (Figure 6). Although longitude, average annual precipitation (Bio1), and average annual temperature range (Bio3) showed no significant direct correlations with genetic parameters, the combined analytical results indicated that altitude, latitude, and annual average temperature collectively shaped the genetic variation observed in *N. incisum* (Figure 6).

Mantel tests revealed that genetic differentiation among populations was not significantly associated with longitude, altitude, or geographic distance. However, a significant but weak pattern of isolation-by-latitude was detected ($R^2 = 0.0237$, $p = 0.03$) (Figure 7). For climatic variables, no significant isolation-by-environment was detected for average annual precipitation, annual average temperature, or annual average temperature range (Figure 8).

Redundancy analysis (RDA) was used to partition the effects of geographical and climatic factors on genetic variation (Table 7, Figure 9). For geographical factors,

TABLE 5 Analysis of molecular variance (AMOVA) of the 38 *Notopterygium incisum* populations.

Source of variation	df	SS	CV	Percentage variation
All populations				
Among populations	37	2534.773	2.534	20.01
Within populations	305	14,766.667	22.788	79.99
Sum	342	17,301.440	25.322	100.00
Two clusters				
Between clusters	1	256.975	0.711	2.77
Within cluster	341	17,044.466	24.919	97.23
Sum	342	17301.440	25.322	100.00

Abbreviations: CV, coefficient of variation; df, degree of freedom; SS, sum of variances.

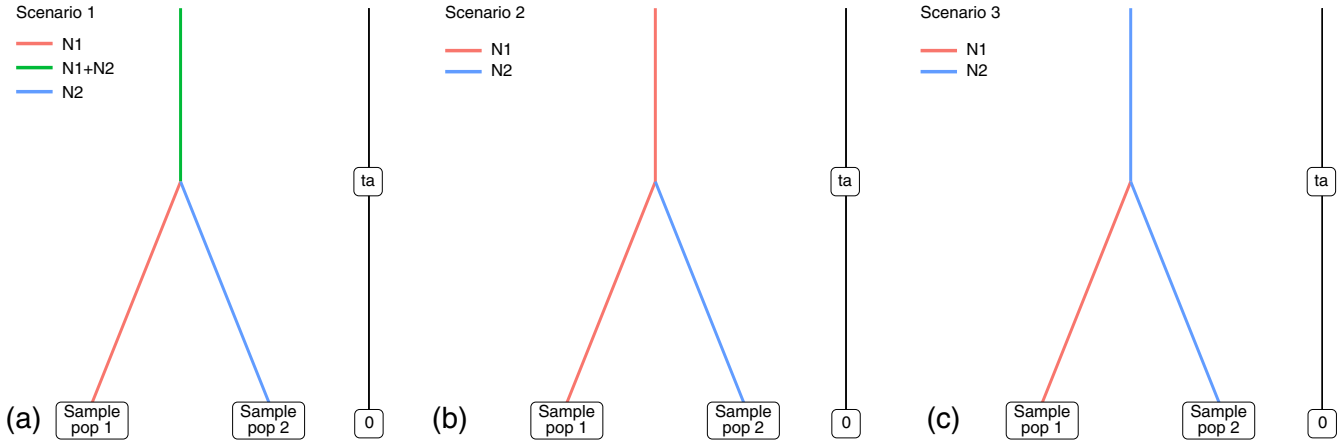


FIGURE 4 Three historical demographic scenarios tested for two genetic clusters of *Notopterygium incisum* using Do-It-Yourself Approximate Bayesian Computation (DIYABC). (a) Scenario 1; (b) Scenario 2; (c) Scenario 3.

TABLE 6 Posterior distributions of the estimated parameters under the best-supported scenario (Scenario 1).

	Lower_bound	Medium	Upper_bound
N1	43	5473	9823
N2	456	4949	9715
t_a	686	4976	9598
μ	1.31e-04	5.60e-04	9.79e-04
P	1.02e-01	1.99e-01	2.91e-01

Note: N1, ancestral population; N2, derived population; t_a, divergence time; μ, mutation rate (per generation per locus); *P*, proportion of multiple-step mutations in the generalized stepwise model.

altitude was identified as a significant predictor ($p = 0.034$). Among climatic variables, average annual temperature (Bio2) significantly explained the genetic variation ($p = 0.039$). The relative lengths of the arrows in the RDA biplot further indicated that altitude and Bio2 were the predominant factors influencing the genetic diversity of *N. incisum* (Figure 9).

4 | DISCUSSION

4.1 | Assessment of genetic diversity in *N. incisum* and implications for conservation

Evaluating genetic diversity in medicinal plant germplasm is essential for elucidating the mechanisms underlying species endangerment and formulating effective conservation strategies to prevent the loss of genetic resources. Such assessments also provide valuable insights for selecting parental materials in breeding programs aimed at enhancing specific traits, particularly for species such as *N. incisum* in the Northwest Sichuan Plateau region of China. Molecular markers represent a well-established tool for investigating plant genetic diversity (Jiang et al., 2018; Pandita et al., 2023; Yang et al., 2019). Studies on species including *Andrographis paniculata* and *Crepidium acuminatum* have demonstrated that different marker systems vary in efficiency, with ISSR markers recognized for their high

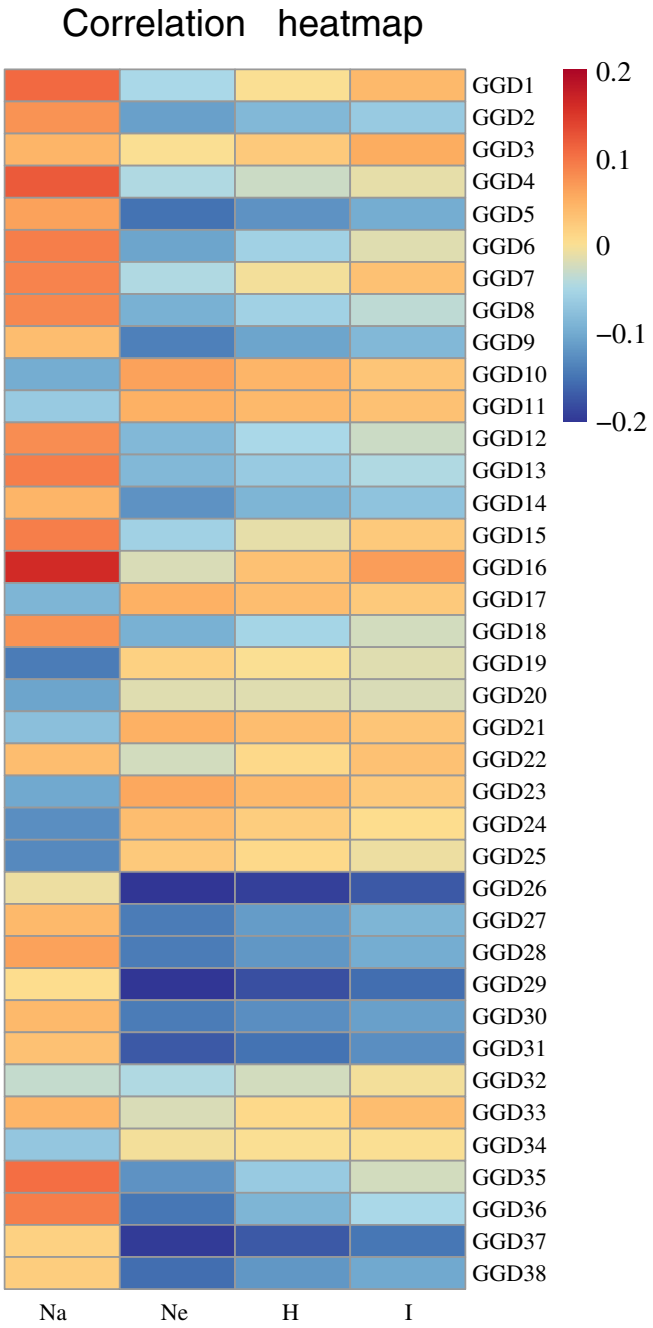


FIGURE 5 Correlation heatmap between geographical distance and genetic diversity parameters. GGD, The geographical distance between each population and all other populations.

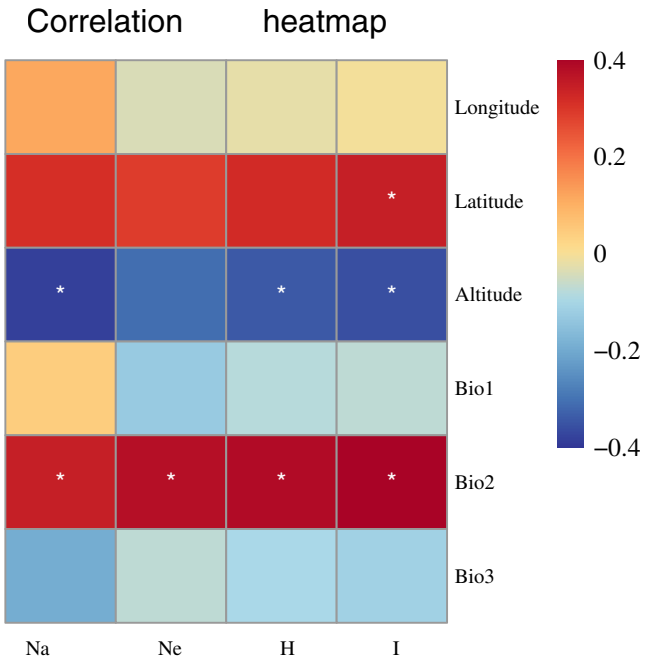


FIGURE 6 Correlation heatmap between environmental factors and genetic diversity parameters of *Notopterygium incisum*. Bio1: average annual precipitation; Bio2: average annual temperature; Bio3: average annual temperature range.

polymorphism and strong discriminatory power (Amom et al., 2020; Thakur et al., 2021; Tiwari et al., 2016; Yang et al., 2019).

Genetic diversity parameters serve as key indicators for assessing the richness of genetic variation within populations. For example, the number of different alleles (*Na*) reflects the degree of polymorphism, while higher values of the effective number of alleles (*Ne*), *Nei*'s genetic distance (*H*), and Shannon's information index (*I*) are associated with greater genetic diversity and enhanced potential for environmental adaptation (Chen et al., 2022). It is generally established that endemic, endangered, and narrowly distributed species tend to exhibit reduced genetic diversity (Godt et al., 1996; Mandel, 2010). In contrast, previous studies on *N. incisum* have reported relatively high levels of genetic diversity across its populations (Jia et al., 2017). Consistent with this, the present study found mean values of $H = 0.2381$ and $I = 0.3603$ for *N. incisum*, which are higher than those reported for *Horsfieldia hainanensis* ($H = 0.1843$, $I = 0.2615$; Jiang et al., 2018) and *Tetrastigma hemsleyanum* ($H = 0.1637$, $I = 0.2403$; Peng et al., 2015), though lower than those of *Ferula pseudalliacea* ($H = 0.29$, $I = 0.45$; Jafari et al., 2019). These comparative results indicate that despite its endemic and endangered status, *N. incisum* maintains a moderate level of genetic diversity.

Although *N. incisum* has a narrow geographical distribution, its populations occur across a substantial altitudinal gradient ranging from 1700 m to 4500 m. The maintenance of a moderate level of genetic diversity in this species is likely an adaptive response to the heterogeneous environmental conditions across this elevational range. This observation is consistent with studies on other endemic and endangered species, such as *Hertia cheirifolia* (Najoua et al., 2022) and *Osmanthus serrulatus*

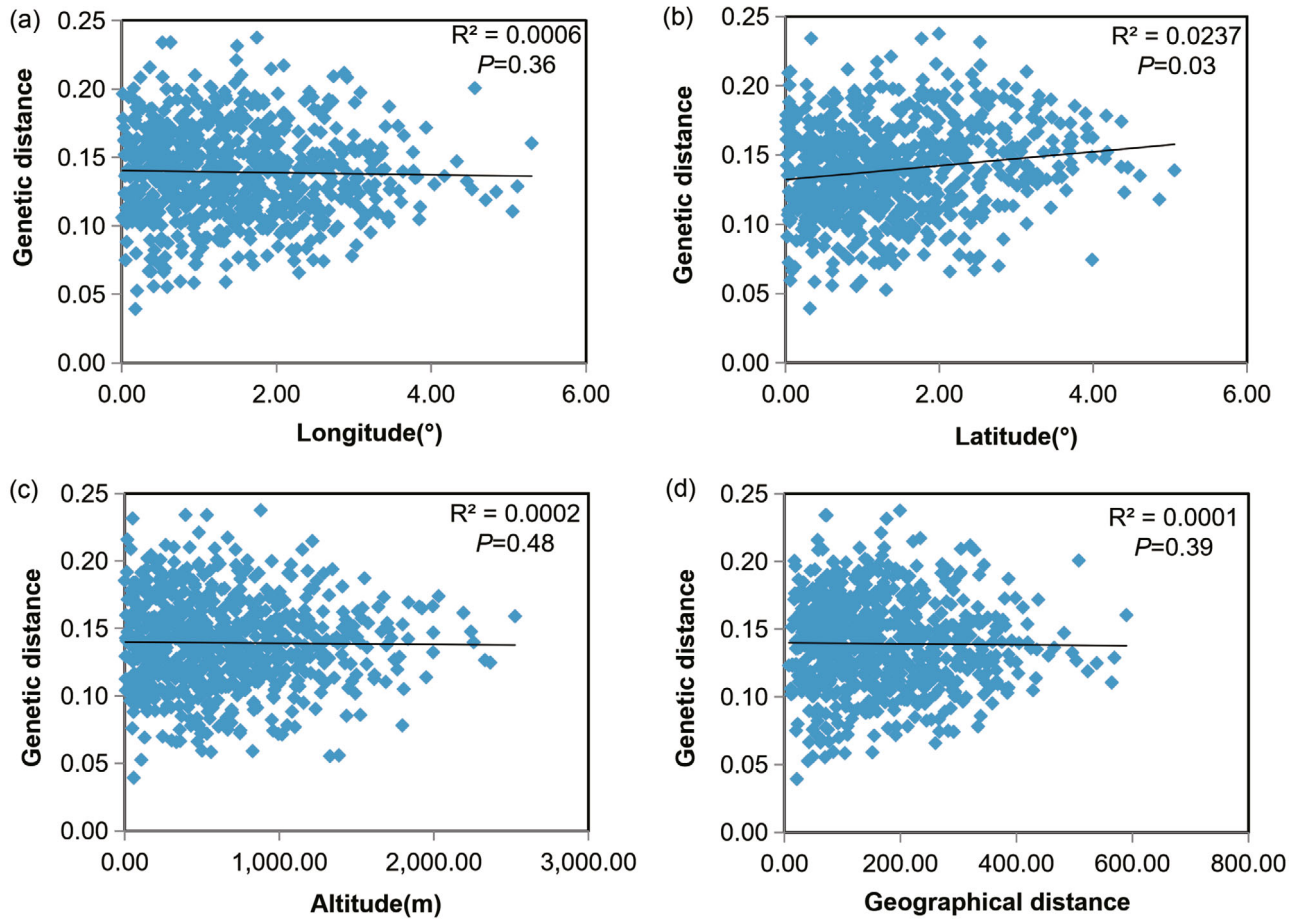


FIGURE 7 Mantel test evaluating the relationship between genetic diversity and geographical factors in *Notopterygium incisum*. *P*, significance level of the correlation, R^2, effect size (strength of the correlation).

(Chen et al., 2021), which also retain moderate to high genetic diversity. Multiple factors can shape genetic diversity patterns, including environmental heterogeneity (Gai et al., 2021) and mating systems (Wu et al., 2022). As an outcrossing species, *N. incisum* inherently maintains higher genetic diversity than self-pollinating plants. Consequently, despite facing challenges such as habitat fragmentation, seed double dormancy, low natural germination rates, and a notable decline in natural population sizes, its outcrossing reproductive strategy has likely been instrumental in preserving sufficient genetic variation to sustain environmental adaptability (Liu et al., 2019).

Based on the genetic diversity parameters assessed across different populations, populations pop1, pop3, pop4, pop5, pop6, pop8, pop9, pop10, pop11, pop12, pop13, pop17, pop18, and pop19 exhibited lower levels of genetic diversity. With the exception of pop4, pop17, and pop18, the lower genetic diversity in the remaining populations is consistent with the general pattern observed in this study that lower latitudes are associated with reduced genetic diversity. The genetic diversity of *N. incisum* was also found to be negatively correlated with altitude. In line with this, pop4—located at a high altitude of 3722 m—showed low genetic diversity. In the case of pop17 and pop18, although situated at higher latitudes and lower altitudes, their genetic diversity may have been reduced due to strong anthropogenic pressure and overexploitation, leading to severe population decline.

Population pop5 displayed the lowest genetic diversity among all studied populations, which may be attributed to its high-altitude distribution, a cold plateau climate, and an annual average temperature below 0°C. This finding aligns with the positive correlation observed in this study between genetic diversity and average annual temperature. In contrast, populations pop7, pop20, pop21, pop25, pop27, pop28, pop29, pop30, pop31, pop33, pop34, pop35, pop36, and pop38 showed relatively high genetic diversity, suggesting enhanced adaptive potential. Except for pop7, all these populations are distributed in higher-latitude regions, consistent with the observed latitudinal gradient in genetic diversity. Although pop7 is located at a lower latitude, its relatively moderate altitude (3179 m) may help explain its higher genetic diversity, possibly

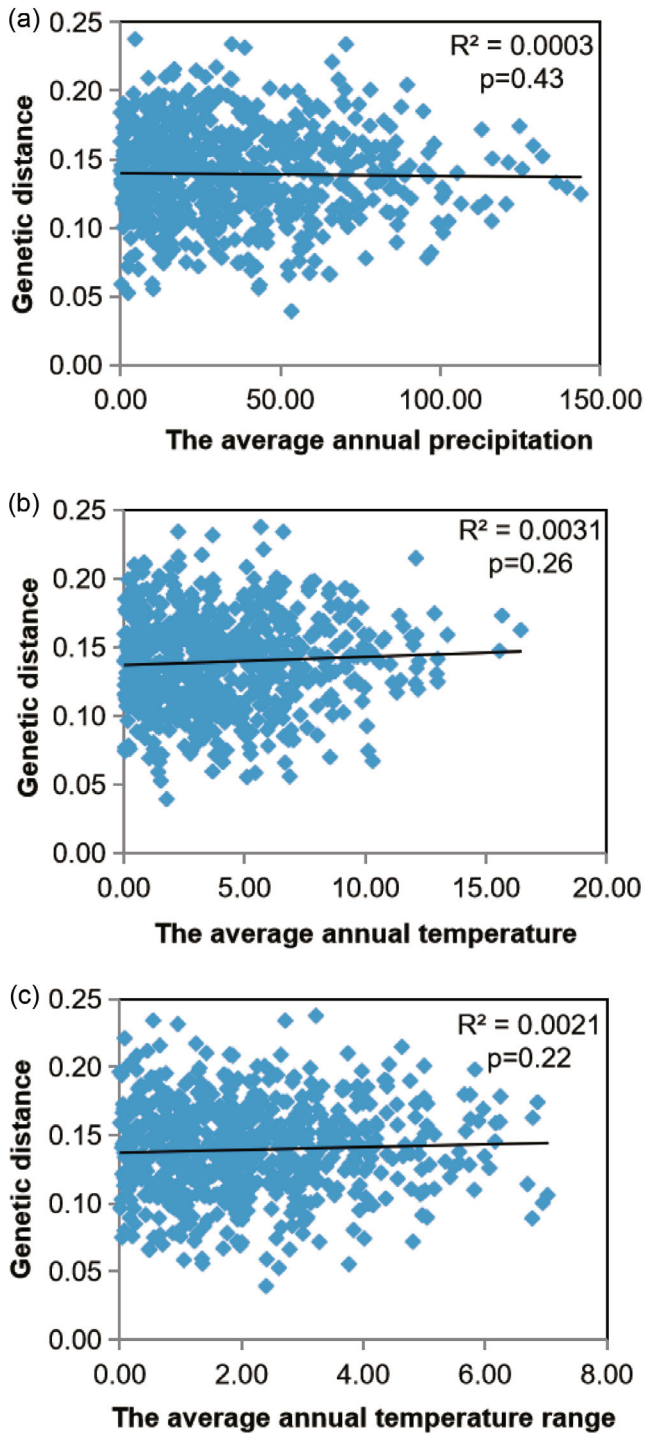


FIGURE 8 Mantel test assessing the relationship between genetic diversity and climatic variables in *Notopterygium incisum*. *P*, significance level of the correlation, R^2, effect size (strength of the correlation).

reflecting the combined influence of altitudinal and climatic factors.

Notopterygium incisum is primarily distributed along the eastern peripheral regions of the Qinghai–Tibet Plateau, encompassing high-altitude areas in northwestern Sichuan, southern Gansu, and northeastern Qinghai. Projections of suitable habitats under future climatic conditions indicate that the species' range will remain largely concentrated on the eastern Qinghai–Tibet Plateau (Zhao et al., 2020). This suggests that Sichuan Province will continue to be a core area for *N. incisum* distribution.

Based on these findings, we recommend integrated in situ and ex situ conservation strategies to preserve the genetic resources of *N. incisum* (Moritz, 2002; Wang et al., 2014). Populations with high genetic diversity—pop7, pop20, pop21, pop25, pop27, pop28, pop29, pop30, pop31, pop33, pop34, pop35, pop36, and pop38—should be prioritized for in situ conservation. Under regulated conditions, these local genetic resources could also support sustainable utilization.

For populations with low genetic diversity, tailored conservation measures are necessary. For instance, pop5 (Seda County, Ganzi, Sichuan; 4184 m altitude) exhibits the lowest genetic diversity, likely due to stressful high-altitude conditions. In such cases, genetic restoration through assisted gene flow—introducing individuals from diverse genetic backgrounds—may enhance diversity. Complementary ex situ conservation, using seed or rhizome banks, can help preserve unique alleles and provide material for future germplasm innovation and potential reintroduction. By contrast, pop17 and pop19 occur in topographically complex regions expected to harbor high diversity, yet show reduced genetic variation, likely resulting from intense human disturbance and overharvesting. For these populations, controlling anthropogenic pressure and reinforcing wild populations with introduced individuals are urgent priorities. In situ protection remains essential to facilitate natural recovery. Through these targeted strategies, the rich genetic heritage of *N. incisum* can be effectively conserved to support its long-term survival and adaptive potential.

4.2 | Genetic differentiation and population genetic structure

Notopterygium incisum is not a hybrid species (Jia et al., 2023). Genetic differentiation describes how genetic diversity is partitioned within and among populations (Jiang et al., 2018). According to established thresholds, *Gst* values indicate low ($0.00 < Gst < 0.05$), moderate ($0.05 < Gst < 0.50$), or high ($Gst > 0.50$) levels of genetic differentiation (Thormann et al., 1994). In the present study, the overall genetic differentiation among the 38 *N. incisum* populations was moderate ($Gst = 0.2764$), with the majority of genetic variation originating from within populations rather than among them. This pattern is commonly associated with outcrossing mating systems,

TABLE 7 Redundancy analysis (RDA) of the relationship between genetic variation and environmental factors in *Notopterygium incisum*.

Environmental factors	RDA1	RDA2	R^2	*p*
Longitude	0.627	−0.780	0.041	0.481
Latitude	0.984	−0.176	0.075	0.268
Altitude	−0.969	0.246	0.167	0.034
Bio1	−0.663	−0.748	0.022	0.675
Bio2	0.999	−0.045	0.161	0.039
Bio3	−0.39	0.345	0.0311	0.554

Note: RDA1 and RDA2, the first and second axes extracted from the redundancy analysis. R^2 (goodness-of-fit), the proportion of total variance in the species community (response variables) that is explained by the constraining environmental variables (explanatory variables). *p*, the statistical significance of the overall RDA model.

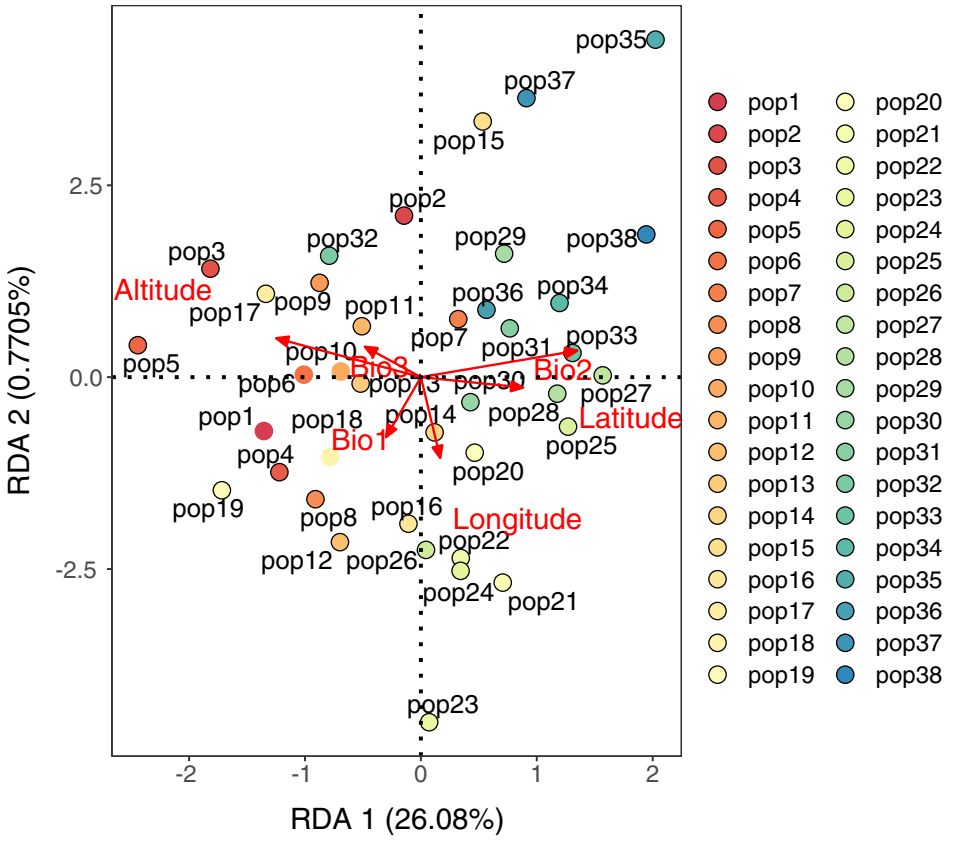


FIGURE 9 Redundancy analysis (RDA) biplot showing the correlation between genetic variation and environmental factors in *Notopterygium incisum*. RDA1 and RDA2, the first and second axes extracted from the redundancy analysis.

which promote gene flow and maintain high levels of intra-population genetic variation (Pandita et al., 2023), as has been reported in species such as *Camellia oleifera* (Zhang et al., 2018) and *Rheum tanguticum* (Hu et al., 2010).

Although UPGMA clustering and PCA separated the 38 populations into two distinct groups (Cluster I and Cluster II), the genetic differentiation between these clusters was low ($Gst = 0.0270$). According to MaxEnt modeling, the distribution range of *N. incisum*—a cold- and drought-tolerant perennial herb—expanded northward from the Last Interglacial (LIG) to the Last Glacial Maximum (LGM) (Shahzad et al., 2017). This expansion aligns with DIYABC results, indicating that both clusters diverged from a common ancestor. Despite subsequent geographic separation by mountain-valley terrain, the species has retained ancestral genetic features, maintaining moderate genetic diversity and low divergence. High gene flow ($Nm > 1$) among populations and between clusters further supports ongoing genetic connectivity, a pattern consistent with post-glacial rapid expansion observed in species such as *Vitex rotundifolia* (Sun

et al., 2019). In summary, although *N. incisum* shows clear genetic structure, it has not undergone significant lineage divergence, and there is no evidence of incipient subspecies formation.

4.3 | Effect of environmental factors on the genetic diversity and medicinal quality of *N. incisum*

The genetic diversity of natural plant populations is influenced by external factors such as altitude and climate (Lyam et al., 2018; Xi et al., 2021). These factors do not act in isolation; rather, their interactions create complex environmental gradients that collectively shape genetic variation within plant populations. In the case of *N. incisum*, genetic diversity has been significantly correlated with latitude, altitude, and precipitation (Yang et al., 2019), while species distribution is constrained by variables including altitude, average annual temperature, slope aspect, and forest type. Future habitat suitability projections further suggest that the species will remain concentrated in the eastern part of the plateau, with Sichuan Province as a core distribution area (Zhao et al., 2020).

This study confirms that latitude, altitude, and average annual temperature are key environmental factors affecting genetic variation in *N. incisum*. Specifically, genetic diversity decreases with altitude but increases with latitude and average annual temperature, consistent with previous findings. Future research should continue to explore the integrated effects of multiple environmental variables on genetic variation.

Beyond genetic diversity, the bioactive components of medicinal plants are also strongly influenced by environmental conditions. The medicinal properties and efficacy of traditional Chinese medicine are embodied in the quality of the crude material, which is in turn affected by a range of external factors such as temperature, humidity, precipitation, wind, topography, soil, and microorganisms (Tang et al., 2010). As sessile organisms, plants must adapt to local environmental conditions, which consequently shape the biosynthesis and accumulation of secondary metabolites. Understanding this process is a central aim of pharmacobotanical research, as it helps elucidate the underlying molecular genetic mechanisms (Colinas & Goossens, 2018). A representative example is *Salvia yunnanensis*, in which the accumulation of its principal components, tanshinones and phenolic acids, is influenced by environmental factors such as rainfall and altitude (Yang et al., 2025).

As a genuine regional medicinal herb (authentic herb), the chemical composition of *N. incisum* is shaped by multiple environmental factors in Sichuan. Studies indicate that notopterol content is strongly positively correlated with sunshine duration, followed by annual precipitation and altitude, whereas isoimperatorin shows a strong negative correlation with annual precipitation and is also influenced by temperature. Sunshine duration, altitude, and precipitation are thus key factors affecting chemical composition in *N. incisum*, with increased sunlight generally promoting compound accumulation and, within a certain range, higher altitude and precipitation enhancing notopterol content (Huang et al., 2013).

Our results demonstrate that the genetic diversity of *N. incisum* is associated with latitude, altitude, and average annual temperature—factors that also influence the formation of its active compounds. The development of authentic herbs involves dual selection by both genetic predisposition and environmental conditions (Sun et al., 2003). We speculate that the scattered distribution of *N. incisum*, subject to diverse environmental pressures, has led to variability in active compound profiles. Through long-term natural selection, this process has contributed to the formation of its unique medicinal quality. Environmental factors thus not only shape the genetic diversity of *N. incisum* but are also crucial in optimizing the formation of its efficacious components, reflecting a complex interplay that collectively determines its medicinal quality and clinical efficacy. Consequently, ensuring consistent drug quality will require the selection of superior varieties coupled with optimal cultivation sites.

5 | CONCLUSIONS

This study represents the first comprehensive analysis of genetic diversity and environmental influences on the endangered medicinal plant *N. incisum* in the southeastern Qinghai–Tibetan Plateau using 15 ISSR markers. The results reveal that *N. incisum* maintains a moderate level of genetic diversity. However, genetic differentiation among populations remains relatively low, indicating no significant trend toward subspecies formation. Populations exhibiting high genetic diversity should be prioritized for in situ conservation. For those with reduced diversity, tailored measures—such as assisted gene flow from genetically distinct sources, complementary ex situ conservation via seed or rhizome banks, reduction of anthropogenic pressure, and population reinforcement—are recommended. These targeted strategies will help preserve the genetic heritage of *N. incisum*, supporting its long-term survival and adaptive capacity. The genetic diversity of *N. incisum* was significantly correlated with latitude, altitude, and average annual temperature.

These findings provide a theoretical foundation for the artificial cultivation and selective breeding of this species. By leveraging diverse genetic resources, breeders may more efficiently achieve desired agronomic or medicinal traits.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, writing—original draft: Jinqiu Liao, Ao Luo, and Rui Zeng; investigation, formal analysis: Rui Zeng, Ao Luo, and Xuexue Deng; data curation, validation, visualization: Jinqiu Liao, Rui Zeng, Ao Luo, Yuanyuan Jiang, and Ruiwu Yang; resources, project administration: Xianhong Feng; writing—review and editing: Jinqiu Liao, Rui Zeng, and Ao Luo; supervision, funding acquisition: Li Zhang and Ruiwu Yang.

FUNDING INFORMATION

This work was supported by the National Modern Agricultural Industry Technology System Sichuan innovation team (grant number SCCXTD-2020-19), Featured Medicinal Plants Sharing and Service Platform of Sichuan Province Funding (2112129170), Sichuan Agricultural University Funding (2021993044), Sichuan Science and Technology Program (2025ZNSFSC0613), and Sichuan Agricultural University (2024ZYTS002).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data supporting the findings of this study are available within the paper and within its Supporting Information published online.

REFERENCES

Amom, T., Tikendra, L., Apana, N., Goutam, M., Sonia, P., Koijam, A. S., Potshangbam, A. M., Rahaman, H., & Nongdam, P. (2020). Efficiency of RAPD, ISSR, iPBS, SCoT and phytochemical markers in the genetic relationship study of five native and economical important bamboos of north-east India. *Phytochemistry*, *174*, 112330. https://doi.org/10.1016/j.phytochem.2020.112330

Azietaku, J. T., Ma, H., Yu, X. A., Li, J., Oppong, M. B., Cao, J., An, M., & Chang, Y. X. (2017). A review of the ethnopharmacology, phytochemistry and pharmacology of *Notopterygium incisum*. *Journal of Ethnopharmacology*, *202*, 241–255. https://doi.org/10.1016/j.jep.2017.03.022

Chen, J., Liu, Q., Lu, C., Liu, Q., Pan, J., Zhang, J., & Dong, S. (2022). Genetic diversity of *Prunus armeniaca* L. var. *ansu* Maxim. germplasm revealed by simple sequence repeat (SSR) markers. *PLoS One*, *17*(6), e0269424. https://doi.org/10.1371/journal.pone.0269424

Chen, L., Pan, T., Qian, H., Zhang, M., Yang, G., & Wang, X. (2021). Genetic diversity and population structure revealed by SSR markers on endemic species *Osmanthus serrulatus* Rehder from southwestern Sichuan Basin, China. *Forests*, *12*(10), 1365. https://doi.org/10.3390/f12101365

Chinese Pharmacopoeia Committee. (2020). *Pharmacopoeia of the People's Republic of China, (Part 1), 190*. China Medical Science and Technology Press.

Colinas, M., & Goossens, A. (2018). Combinatorial transcriptional control of plant specialized metabolism. *Trends in Plant Science*, *23*(4), 324–336. https://doi.org/10.1016/j.tplants.2017.12.006

Collin, F. D., Durif, G., Raynal, L., Lombaert, E., Gautier, M., Vitalis, R., Marin, J. M., & Estoup, A. (2021). Extending approximate Bayesian computation with supervised machine learning to infer demographic history from genetic polymorphisms using DIYABC random forest. *Molecular Ecology Resources*, *21*(8), 2598–2613. https://doi.org/10.1111/1755-0998.13413

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Gai, Z., Zhai, J., Chen, X., Jiao, P., Zhang, S., Sun, J., Qin, R., Liu, H., Wu, Z., & Li, Z. (2021). Phylogeography reveals geographic and environmental factors driving genetic differentiation of *Populus* sect. *Turanga* in Northwest China. *Frontiers in Plant Science*, *12*, 705083. https://doi.org/10.3389/fpls.2021.705083

Giachino, R. R. A., & Boztaş, G. (2025). Advanced techniques and applications in fennel (*Foeniculum vulgare* Mill.) breeding. *Molecular Genetics and Genomics*, *300*(1), 85. https://doi.org/10.1007/s00438-025-02294-y

Godt, M. J. W., Johnson, B. R., & Hamrick, J. L. (1996). Genetic diversity and population size in four rare southern Appalachian plant species. *Conservation Biology*, *10*(3), 796–805. https://doi.org/10.1046/j.1523-1739.1996.10030796.x

Hollander, M., Wolfe, D. A., & Chicken, E. (2015). *Nonparametric statistical methods* (Third ed.). John Wiley & Sons.

Hu, Y., Wang, L., Xie, X., Yang, J., Li, Y., & Zhang, H. (2010). Genetic diversity of wild populations of *Rheum tanguticum* endemic to China as revealed by ISSR analysis. *Biochemical Systematics and Ecology*, *38*(3), 264–274. https://doi.org/10.1016/j.bse.2010.01.006

Huang, L. F., Li, W. T., Wang, Z., Fu, J., & Chen, S. L. (2013). Correlative study between chemical constituents and ecological factors of *Notopterygii* rhizoma et radix of endangered plateau plant. *Acta Ecologica Sinica*, *201333*(24), 7667–7678. https://doi.org/10.5846/stxb201209221337

Jafari, S. H., Sepehry, A., Soltanloo, H., & Karimian, A. A. (2019). Genetic differentiation between bitter and sweet asafetida plants using ISSR markers. *Molecular Biology Reports*, *46*(1), 1069–1078. https://doi.org/10.1007/s11033-018-4565-1

Jia, Y., Ba, i. J. Q., Liu, M. L., Jiang, Z. F., Wu, Y., Fang, M. F., & Li, Z. H. (2019). Transcriptome analysis of the endangered *Notopterygium incisum*: Cold-tolerance gene discovery and identification of EST-SSR and SNP markers. *Plant Diversity*, *41*(1), 1–6. https://doi.org/10.1016/j.pld.2019.01.001

Jia, Y., Liu, M. L., López-Pujol, J., Jia, R. W., Kou, Y. X., Yue, M., Guan, T. X., & Li, Z. H. (2023). The hybridization origin of the Chinese endemic herb genus *Notopterygium* (Apiaceae): Evidence from population genomics and ecological niche analysis. *Molecular Phylogenetics and Evolution*, *182*, 107736. https://doi.org/10.1016/j.ympev.2023.107736

Jia, Y., Liu, M. L., Yue, M., Zhao, Z., Zhao, G. F., & Li, Z. H. (2017). Comparative transcriptome analysis reveals adaptive evolution of *Notopterygium incisum* and *Notopterygium franchetii*, two high-alpine herbal species endemic to China. *Molecules*, *22*(7), 1158. https://doi.org/10.3390/molecules22071158

Jiang, Y., Liu, X., Xiang, W., Jiang, Y., & He, Y. (2018). Genetic diversity and structure analysis of the endangered plant species *Horsfieldia hainanensis* Merr. in China. *Biotechnology & Biotechnological Equipment*, *32*(1), 95–101. https://doi.org/10.1080/13102818.2017.1391122

Li, L., Wang, R., Wang, Z., Wang, S., & Li, S. (1996). The studies of Naidong *Camellia* biodiversity in Qingdao (II)—Genetic diversity analysis of populations. *Biodiversity Science*, *4*(1), 1. https://doi.org/10.17520/biods.1996001

Liu, M. L., He, Y. L., López-Pujol, J., Jia, Y., & Li, Z. H. (2019). Complex population evolutionary history of four cold-tolerant *Notopterygium* herb species in the Qinghai-Tibetan Plateau and adjacent areas. *Heredity*, *123*(2), 242–263. https://doi.org/10.1038/s41437-019-0186-2

Lyam, P. T., Duque-Lazo, J., Durka, W., Hauenschild, F., Schnitzler, J., Michalak, I., Ogundipe, O. T., & Muellner-Riehl, A. N. (2018). Genetic diversity and distribution of *Senegalia senegal* (L.) Britton under climate change scenarios in West Africa. *PLoS One*, *13*(4), e0194726. https://doi.org/10.1371/journal.pone.0194726

Mandel, J. R. (2010). Clonal diversity, spatial dynamics, and small genetic population size in the rare sunflower, *Helianthus verticillatus*. *Conservation Genetics*, *11*(5), 2055–2059. https://doi.org/10.1007/s10592-010-0062-3

Moritz, C. (2002). Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology*, *51*(2), 238–254. https://doi.org/10.1080/10635150252899752

Najoua, R., Islem, Y., Mohamed, B., & Chokri, M. (2022). Assessment of genetic diversity and population structure of the endemic *Hertia cheirifolia* (L.) Kuntze based on ISSR and SRAP molecular markers. *Biologia*, *77*(12), 3429–3439. https://doi.org/10.1007/s11756-022-01166-9

Nevo, E., Baum, B. R., Beiles, A., & Johnson, D. A. (1998). Ecological correlates of RAPD DNA diversity of wild barley, *Hordeum spontaneum*, in the Fertile Crescent. *Genetic Resources and Crop Evolution*, *45*(2), 151–159. https://doi.org/10.1023/A:1008616923427

Pandita, D., Mahajan, R., Zargar, S. M., Nehvi, F. A., Dhekale, B., Shafi, F., Shah, M. U. D., Sofi, N. R., & Husaini, A. M. (2023). Trait specific marker-based characterization and population structure analysis in rice (*Oryza sativa* L.) germplasm of Kashmir Himalayas. *Molecular Biology Reports*, *50*(5), 4155–4163. https://doi.org/10.1007/s11033-023-08324-5

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics*, *28*(19), 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Peng, X., Ji, Q., Fan, S., Zhang, Y., & Zhang, J. (2015). Genetic diversity in populations of the endangered medicinal plant *Tetrastigma hemsleyanum* revealed by ISSR and SRAP markers: Implications for conservation. *Genetic Resources and Crop Evolution*, *62*(7), 1069–1078. https://doi.org/10.1007/s10722-014-0210-6

Semagn, K., Bjornstad, A., Stedje, B., & Bekele, E. (2000). Comparison of multivariate methods for the analysis of genetic resources and adaptation in *Phytolacca dodecandra* using RAPD. *Theoretical and Applied Genetics*, *101*(7), 1145–1154. https://doi.org/10.1007/s001220051591

Shahzad, K., Jia, Y., Chen, F. L., Zeb, U., & Li, Z. H. (2017). Effects of mountain uplift and climatic oscillations on phylogeography and species divergence in four endangered *Notopterygium* herbs. *Frontiers in Plant Science*, *8*, 1929. https://doi.org/10.3389/fpls.2017.01929

She, M., & Watson, M. F. (2005). Notopterygium. In Z. Y. Wu & P. H. Raven (Eds.), *Flora of China*. Science Press.

Sun, K., Zhang, H., Chen, W., Chen, X. L., & Ma, R. J. (2003). Review on the geo-herbalism of medicinal plant by DNA molecular markers. *Journal of Northwest Normal University (Natural Science)*, *03*, 100–103. https://doi.org/10.16783/j.cnki.nwnuz.2003.03.030

Sun, Y., Yang, H., Zhang, Q., Qin, L., Li, P., Lee, J., Chen, S., Rahman, K., Kang, T., & Jia, M. (2019). Genetic diversity and its conservation implications of *Vitex rotundifolia* (Lamiaceae) populations in East Asia. *PeerJ*, *7*, e6194. https://doi.org/10.7717/peerj.6194

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–2729. https://doi.org/10.1093/molbev/mst197

Tang, S. H., Yang, H. J., & Huang, L. Q. (2010). Discuss on effect of physical environmental factors on nature of Chinese materia medica. *China Journal of Chinese Materia Medica*, *35*(1), 126–128. https://doi.org/10.4268/cjcmm20100127

Tang, X. F., Jiang, S. Y., Sun, H., Chen, T. Z., Zhou, Y., & Ma, X. J. (2008). RAPD analysis of genetic diversity of *Notopterygium incisum* in Sichuan. *Chinese Traditional and Herbal Drugs*, *39*(9), 1387–1392. https://doi.org/10.3321/j.issn:0253-2670.2008.09.032

Ter Braak, C. J. F. (1988). *CANOCO—A fortran program for canonical community ordination by [Partial] [Detrended] [Canonical] correspondence analysis, principal components analysis and redundancy analysis (version 2.1). Technical Report. LWA-88-02, GLW, 95*. Agricultural Mathematics Group.

Thakur, J., Dwivedi, M. D., Singh, N., Uniyal, P. L., Goel, S., & Pandey, A. K. (2021). Applicability of start codon targeted (SCoT) and inter simple sequence repeat (ISSR) markers in assessing genetic diversity in *Crepidium acuminatum* (D. Don) Szlach. *Journal of Applied Research on Medicinal and Aromatic Plants*, *23*, 100310. https://doi.org/10.1016/j.jarmap.2021.100310

Thormann, C. E., Ferreira, M. E., Camargo, L. E., Tivang, J. G., & Osborn, T. C. (1994). Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. *Theoretical and Applied Genetics*, *88*(8), 973–980. https://doi.org/10.1007/BF00220804

Tiwari, G., Singh, R., Singh, N., Choudhury, D. R., Paliwal, R., Kumar, A., & Gupta, V. (2016). Study of arbitrarily amplified (RAPD and ISSR) and gene targeted (SCoT and CBDP) markers for genetic diversity and population structure in Kalmegh [*Andrographis paniculata* (Burm. f.) Nees]. *Industrial Crops and Products*, *86*, 1–11. https://doi.org/10.1016/j.indcrop.2016.03.031

Wang, D. Y., Chen, Y. J., Zhu, H. M., Lv, G. S., Zhang, X. P., & Shao, J. W. (2014). Highly differentiated populations of the narrow endemic and endangered species *Primula cicutariifolia* in China, revealed by ISSR and SSR. *Biochemical Systematics and Ecology*, *53*, 59–68. https://doi.org/10.1016/j.bse.2013.12.025

Wang, S., & Xie, Y. (2005). *China species red list (first volumes)*. Higher Education Press.

Wu, Z. Y., & Raven, R. H. (2005). Notopterygium. In Z. Y. Wu & P. H. Raven (Eds.), *Flora of China*. Science Press.

Wu, Z., Wang, Z., Xie, D., Wang, H., Zhao, A., Wang, Y., Wang, H., Xu, X., Li, T., & Zhao, J. (2022). Effects of highland environments on clonal diversity in aquatic plants: An interspecific comparison study on the Qinghai-Tibetan Plateau. *Frontiers in Plant Science*, *13*, 1040282. https://doi.org/10.3389/fpls.2022.1040282

Xi, F., Ruan, X., & Amir, A. M. (2021). ISSR markers and population differentiations in *Erodium ciconium* (L.) L'hér ex Aition. *Bangladesh Journal of Plant Taxonomy*, *28*(1), 61–73. https://doi.org/10.3329/bjpt.v28i1.54208

Yang, L. C., Liu, H. C., Zhou, X. L., Xu, W. H., & Zhou, G. Y. (2015). ITS sequences variation and phylogenetic analysis on 31 geographical populations of *Notopterygium incisum*. *China Journal of Chinese Materia Medica*, *40*(19), 3748–3753. https://doi.org/10.4268/cjcmm20151910

Yang, L., Lu, G., Zhao, Y., & Zhou, G. (2019). Effects of environmental factors on the population genetic diversity in the Chinese endangered and endemic medicinal plant *Notopterygium incisum*. *Pakistan Journal of Botany*, *51*(5), 1783–1791. https://doi.org/10.30848/PJB2019-5(22)

Yang, X., Zhang, Q., Xi, e. J., Su, S., Zhao, L., Wang, T., Su, J., & Zhang, Y. (2025). A comprehensive review on the phytochemistry, pharmacological activities, geographical distribution and genetic relationships of *Salvia yunnanensis*. *Fitoterapia*, *186*, 106816. https://doi.org/10.1016/j.fitote.2025.106816

Yeh, F., Yang, R., & Boyle, T. (1999). *POPGENE version 1.32 Microsoft Windows-based freeware for populations genetic analysis*. Molecular biology and biotechnology Centre, University of Alberta.

Zhang, S., Wang, S., Wang, S., Su, H., & De, J. (2025). Genetic diversity and metabolic profile of Tibetan medicinal plant *Saussurea obvallata*. *Genes*, *16*(5), 593. https://doi.org/10.3390/genes16050593

Zhang, Z., Meng, J., Pan, D., Yang, C., & Li, Y. (2018). Mating system and progeny genetic diversity of *Camellia oleifera* 'Ruan Zhi'. *Journal of Forestry Research*, *30*(5), 1805–1810. https://doi.org/10.1007/s11676-018-0732-4

Zhao, Z., Guo, Y., Wei, H., Ran, Q., Liu, J., Zhang, Q., & Gu, W. (2020). Potential distribution of *Notopterygium incisum* Ting ex H. T. Chang and its predicted responses to climate change based on a comprehensive habitat suitability model. *Ecology and Evolution*, *10*(6), 3004–3016. https://doi.org/10.1002/ece3.6117

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Liao, J., Luo, A., Zeng, R., Feng, X., Jiang, Y., Deng, X., Zhang, L., & Yang, R. (2026). Effects of environmental factors on genetic diversity in the endangered *Notopterygium incisum* from the southeastern Qinghai–Tibetan Plateau. *Plant Species Biology*, *41*(2), e70047. https://doi.org/10.1111/1442-1984.70047