

Analysis of genetic diversity and population structure in *Polygonatum cyrtoneura* Hua from China using SSR markers

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Abstract

Polygonatum cyrtoneura Hua is a perennial herb of the Liliaceae family, which is a traditional medicinal and dietary herb in China. The large-scale introduction of cultivation, asexual reproduction and geographical differences have caused confusion in germplasm resources and variation in plant morphology. Therefore, based on the phenotypic trait identification analysis, 96 *Polygonatum cyrtoneura* Hua germplasm were used as materials to analyze the genetic diversity and genetic structure of germplasm resources from seven different regions using SSR molecular marker technology. Ten polymorphic primer pairs were screened from 50 pairs of SSR primers, and a total of 70 alleles were detected, with an average of 7 alleles per locus. The polymorphic information content (PIC) generated ranged from 0.40–0.86 with a mean value of 0.72. Genetic structure and clustering analyses divided *Polygonatum cyrtoneura* Hua into three populations (JZ population, QY population, and JD population). The genetic diversity analysis showed that the population mean allele number, observed heterozygosity (H_o), expected heterozygosity (H_e), and Shannon's index (I) were 6.90, 0.46, 0.57, and 1.08, respectively. The three populations were genetically differentiated from each other. The plants of the three populations have been changed from morphology to genetics. In this study, the rich genetic diversity of *Polygonatum cyrtoneura* Hua was detected with fewer polymorphic primers, indicating the unique advantage of SSR marker technology in the analysis of genetic diversity of closely related species, and providing a reference for scientific management of *Polygonatum cyrtoneura* Hua germplasm resources and molecular marker-assisted breeding.

1. Introduction

There are about 40 species of *Polygonatum* in the world, mainly distributed in the northern temperate zone, and distributed in Europe, Asia, North America and other regions. China has more than 30 species of *Polygonatum* (Editorial Committee of Flora of China 2000), which is the distribution center of *Polygonatum*. *Polygonatum cyrtoneura* Hua is a perennial herb of the *Polygonatum* in the Liliaceae family and is one of the three medicinal *Polygonatum* Rhizoma stipulated in the "Pharmacopoeia of the People's Republic of China" (Chinese Pharmacopoeia Commission. 2020). *P. cyrtoneura* has been used as medicine in China for more than 2,000 years and is a traditional medicinal herbal medicine with food. In addition to its medicinal values such as anti-aging, immune modulation, blood lipid regulation, memory improvement, anti-tumor, and antibacterial (Zhao 2005, Wu 2014, Huang 2016, Li 2018, Yang 2018, Li 2019, Ma 2019, Fan ZW 2020), *P. cyrtoneura* also has broad prospects for development in food and health products.

P. cyrtoneura is distributed in all provinces of the Yangtze River basin in China, of which Anhui is one of its main production areas. According to the "Flora of Anhui" (Group 1992), *P. cyrtoneura* is distributed in Qimen County, Shitai County, Xiuning County,, Tongling City, and Jinzhai County, which can be divided into two major regions, Dabie Mountain and South Anhui Mountain.

Previous studies on the germplasm resources and plant morphological characteristics of *P. cyrtanema* (Cheng 2021) revealed that there were obvious differences in plant organs such as rhizomes, leaves and pedicels between Dabie and South Anhui Mountainous areas. Based on the differences in plant morphology and distribution areas, *P. cyrtanema* can be divided into three major taxa. In the Dabie Mountain area (JZ population) the plants are tall overall, with long total pedicels, often more than twice as long as the pedicels, the rhizomes are often cylindrical or ginger-shaped, with larger stem scars, often accounting for more than half of the rhizomes, and more winged buds. The plants in the mountainous areas of southern Anhui (QY population and JD population) are small compared with the JZ population, and the rhizomes are checkerboard-shaped. The QY population plants have smaller stem scars and the JD population plants have more than two to three shoots per year on the rootstock. Further studies on the fitness zones and ecological factors of *P. cyrtanema* revealed that both Dabie and South Anhui Mountains are highly suitable zones for *P. cyrtanema*, and the dominant ecological factors causing differences in plant morphology between the two regions are eight factors such as precipitation in February and March, soil type, vegetation type, and slope orientation.

In the study of diversity of plant morphological characters, phenotypic polymorphism is often low, and the accuracy of plant clustering by this means needs to be improved. Therefore, the application of DNA molecular marker technology in the study of genetic diversity of germplasm resources is becoming more and more common, among which ISSR molecular markers and SSR molecular markers are more widely used. Compared with ISSR, SSR has strong specificity and high polymorphism for closely related species, which is suitable for genetic diversity among closely related populations (Li 2005, Zhu 2018, Ji 2020). SSR molecular markers have the advantages of high polymorphism, co-dominant inheritance, simple and rapid technique, and good reproducibility. More importantly, SSR marker technology is not restrictive to the developmental period and organs of plant materials, which makes it easier to study the kinship of species at the molecular level, conserve germplasm resources, construct mapping and assist breeding. Currently, SSR molecular markers have been widely used in the study of genetic diversity, classification, evolutionary origin, fingerprinting and genetic map construction of plant resource (Liu 2010, Feng 2016, Wang 2018, Getaneh 2021, Feng PL 2022, Li 2022, Mao 2022).

In this study, based on the previous study on the diversity of morphological characters of *P. cyrtanema* plants in different regions, 96 *P. cyrtanema* germplasm resources from seven regions were analyzed for genetic diversity using SSR molecular marker technology to elucidate the kinship and population genetic structure of *P. cyrtanema* germplasm resources from different sources and to evaluate the diversity of *P. cyrtanema* germplasm resources. In addition, new germplasm has greater disadvantages in both breeding and resistance to pests and diseases because of the lack of gene flow between species due to directed breeding of new germplasm and the increasingly narrow genetic background. In this study, modern molecular biology techniques were used to study the genetic diversity of the crop, with a view to having important theoretical and practical significance for the resource management and conservation of *P. cyrtanema* germplasm and molecular marker-assisted breeding.

2. Materials And Methods

2.1 Plant Materials

In this study, a total of 96 leaf samples were collected from Jinzhai County, Huoshan County, Qingyang County, Qimen County, Huangshan City and Jingde County areas of Anhui Province, which basically covered the main distribution areas of *P. cyrtoneura*. The original plant was identified on site by Prof. Yu Nianjun and Prof. Liu Helin of Anhui University of Traditional Chinese Medicine as *P. cyrtoneura*, family Liliaceae. More than 10 individuals were collected from each area (the interval between individuals was not less than 10m), and fresh young leaves were collected, dried with silica gel and stored in an ultra-low temperature refrigerator at -70°C for backup. The resource survey and sampling were completed in 2020, and the sample collection information is shown in (Table 1).

Table 1
Information of 96 *Polygonatum cyrtoneura* Hua leaves leaf collections from seven regions

Population code	Geographic location	Longitude	Latitude	Altitude (m)	Sample ID
JZ	Changling Township in Jinzhai County of Anhui Province	E115°56'46.82"	N31°13'48.78"	461	DH1-16
	Taipingfan Township in Huoshan County of Anhui Province	E115°28'09.56"	N31°05'18.48"	435	DH17-32
QY	Youhua Town in Qingyang County of Anhui Province	E117°59'35.21"	N33°38'04.28"	124	DH33-48
	Liyuan Village in Shitai County of Anhui Province	E117°25'17.50"	N30°08'24.33"	221	DH49-64
JD	Baidi Town in Jingde County of Anhui Province	E118°21'59.43"	N30°11'44.39"	263	DH65-81
	Fuxi Village in Huangshan City of Anhui Province	E117°17'19.87"	N30°05'44.39"	592	DH82-90
	Tafang Town in Qimen County of Anhui Province	E117°17'17.07"	N29°56'48.59"	131	DH91-96
JZ: populations in Jinzhai and Huoshan areas,QY: populations in Qingyang and Shitai areas,JD: populations in Qimen, Huangshan, Jingde areas					

2.2 DNA extraction and detection

The total genomic DNA was extracted from the sample leaves by CTAB method. After the quality and concentration were detected by 1% agarose gel electrophoresis, the concentration was adjusted to 50ng/uL and stored in a -20°C refrigerator for later use.

2.3 PCR amplification and SSR primer screening

Based on the relevant studies, 50 pairs of primers were screened in this paper, and the primers were synthesized by Biotech Bioengineering (Shanghai) Co. A total of 14 samples were randomly selected from 2 samples in each of the 7 sample collection areas for PCR amplification pre-experiments. Different loci can be selected for pre-experiments for different samples to ensure coverage of all loci. 3 μ L of amplification product was taken for electrophoresis to identify whether the PCR conditions were single and whether the fragment size was as expected. Bands with a single and consistent size can enter the experiment, and the concentration can be adjusted by adjusting the number of amplification cycles (Fig. 1, Fig. 2). PCR reaction system: 1 μ L of template DNA, 0.5 μ L each of forward and reverse primers ($10 \mu\text{mol.L}^{-1}$), 5 μ L of MIX enzyme, and 10.00 μ L of total system with ddH₂O. Amplification program: pre-denaturation at 96°C for 3 min, (denaturation at 94°C for 1 min, 56°C for 1 min, extension at 72°C for 1 min) * 20 cycles, extension at 60°C for 10 min, and storage at 12°C. The amplified product was stored in a -20°C refrigerator.

The amplification products were diluted to appropriate concentrations and sequenced on the Fragment AnalyzerTM fully automated capillary electrophoresis system, and the Prosize TM2.0 software was applied to score the polymorphic DNA bands in the capillary electrophoresis profiles (Chen 2020). The fragment length, effective allele number (Ne), major allele frequency (MAF), observed heterozygosity (Ho), expected heterozygosity (He) and polymorphic information content (PIC) were calculated using PowerMarker software, and finally 10 pairs of SSR primers with high polymorphism, clear bands and good reproducibility were selected for the population genetic study of *P. cyrtonema*, and the primer details are shown in (Table 2).

Table 2
Information of 10 pairs of polymorphic SSR primers screened

Primer name	Forward primer sequence (5'-3')	Reverse primer sequence (3'-5')	Repeat motifs
DH11	GTCCGAGTTCTTTGACGAGC	AAAACCATCTCCATCCTCCC	(TG) ₁₀ (AG) ₆
DH15	CGAAGAAGTCTCGATCCACC	TCGAGGAGTGCTTGATGATG	(TAACCC) ₄
DH18	TCTGTAAATCAGGAGGAGCGA	AATCGATGAGATCGACCTGG	(CT) ₁₂
DH19	CGAAACCCTCCTCAATCTCA	GCGACAAGTGTTGAAGGGTT	(ACC) ₉
DH21	CCAACAAGGGAAAAGCAAAG	AATATGGGGGTGACTCCGTT	(AGAA) ₆
DH24	ACCTAAAAGCCTCCAGCGAT	AGGAGGAGGAGGATAGGGGT	(CCGTAC) ₄
DH26	ACCCTCTCAATCGTCACCTG	TCAATCAATTCCTTCAGC	(CTG) ₈
DH34	GCTCGCAACAACAAACAAAA	RGGGCTGAGAATTGAGAACCA	(TTAA) ₆
DH38	TGACCCTCCACTTCATCTCC	GACTCCTTCGAGTGGTACGC	(CGG) ₈
DH40	TACTTTCTCCCCGTTTCCCT	GCTCCCCATCTCCAGAAATA	(ACCG) ₆

2.4 Data processing and analysis

The PowerMarker software (Zhang 2012, Lou 2022) was used to calculate indices of allele number (Na), expected heterozygosity (Ho), observed heterozygosity (He), major allele frequency (MAF) and polymorphic information content (PIC) per microsatellite locus, respectively, for genetic diversity analysis.

Allele frequencies were calculated using PowerMaker software, and then UPGMA clustering analysis was performed using NTSYS-PC 2.1 (Falush 2003) software to construct the NJ phylogenetic tree. Next, the genetic structure of 96 *P. cyrtonema* germplasm was analyzed by STRUCTURE software based on Bayesian clustering algorithm. For the calculation, the grouping (K) was set according to the number of populations sampled (Goudet 1995, Tian 2022), the Admixture ancestry model and the Independent Allele Frequencies model were selected, and the Length of burn-in-period and Number of MCMC replication after burn in parameters were both set to 100,000. Set the number of populations K values from 2–10 and repeat each K value 10 times. After the operation is completed, the results of the K-value operation are uploaded to the STRUCTURE HARVESTER (<http://taylor0.biology.ucla.edu/structureHarvester/>) website (Yu 2020), and the best division K value is judged according to the LnP(D) corresponding to the K value and the calculated ΔK value.

AMOVA (analysis of molecular variance) analysis was performed using GenALEX V.6.5 (Lade 2020) to calculate the percentage of individuals between varieties, between populations and within populations,

and to calculate F_{st} and the three population differentiations ϕ_{st} (Bougma 2021) to infer whether there was genetic exchange and genetic differentiation between populations.

3 Results

3.1 SSR primer diversity

50 primer pairs were evaluated using 14 germplasm from seven regions and 10 pairs of SSR primers with good polymorphism were selected and the testing information is shown in (Table 3). Gene frequencies of alleles in different populations (Fig. 3) were detected for a total of 70 alleles, with the number of alleles per locus (N_a) ranging from 4 to 9. The MAF ranged from 0.18–0.70 with a mean value of 0.43. The expected heterozygosity (H_e) ranged from 0.44 to 0.77, with a mean value of 0.60. The observed heterozygosity (H_o) ranged from 0.08 to 0.90, with a mean value of 0.45. The polymorphic information content (PIC) generated ranged from 0.33 to 0.88, with a mean value of 0.64. The mean intrapopulation inbreeding coefficient (F_{is}) was 0.38. The 10 pairs of SSR primer marker loci are all highly polymorphic loci with rich genetic diversity, which are suitable for genetic study of *P. cyrtoneura*.

Table 3
Detection information of 10 pairs of polymorphic SSR primers

Maker	N_a	N_e	I	H_o	H_e	uH_e	MAF	PIC	F_{is}
DH11	4.000	2.027	0.740	0.906	0.507	0.509	0.7083	0.3396	0.792
DH15	8.000	3.805	1.491	0.740	0.737	0.741	0.4323	0.6316	0.068
DH19	7.000	3.367	1.432	0.375	0.703	0.707	0.2813	0.7781	0.416
DH21	8.000	2.185	1.114	0.427	0.542	0.545	0.2344	0.8276	0.178
DH24	9.000	4.358	1.674	0.427	0.771	0.775	0.4063	0.6249	0.356
DH26	7.000	1.784	0.896	0.083	0.440	0.442	0.7083	0.4523	0.807
DH34	8.000	1.908	0.999	0.271	0.476	0.478	0.4323	0.6823	0.429
DH38	7.000	2.954	1.377	0.219	0.662	0.665	0.3750	0.7636	0.654
DH40	5.000	2.122	0.903	0.510	0.529	0.532	0.6094	0.4507	0.041
DH18	7.000	3.102	1.335	0.625	0.678	0.681	0.1823	0.8794	0.038
Mean	7.000	2.761	1.196	0.458	0.604	0.607	0.4370	0.6430	0.378
Na = number of alleles, Ne = number of effective alleles, Ho = observed heterozygosity, I = Shannon's information index, He = expected heterozygosity, uHe = unbiased expected heterozygosity, MAF = major allele frequency, PIC = Polymorphism information content, Fis = Intraspecific inbreeding coefficient									

3.2 Population genetic diversity analysis

The data of genetic diversity analysis of the three *P. cyrtonema* populations after amplification by SSR are shown in (Table 4). The average number of alleles (Na) in 96 germplasm was 4.73, and the average number of alleles in the JZ, QY and JD populations were 4.60, 4.90 and 4.70, respectively, and the average effective number of alleles was 2.43, 2.64 and 2.72, respectively. The JZ population had the lowest number of alleles, indicating that the germplasm of the JZ population was more purified. The number of valid alleles in the JD population was closest to the number of alleles measured, indicating that the alleles were relatively evenly distributed in this population.

Table 4
Data table of genetic diversity analysis of three populations

populations	Na	Ne	I	Ho	He	uHe
JZ	4.600	2.436	1.056	0.428	0.568	0.577
QY	4.900	2.644	1.097	0.481	0.574	0.583
JD	4.700	2.719	1.096	0.466	0.569	0.578
Mean	4.73	2.5	1.08	0.45	0.57	0.58
JZ: populations in Jinzhai and Huoshan areas,QY: populations in Qingyang and Shitai areas,JD: populations in Qimen, Huangshan, Jingde areas						

The mean observed heterozygosity (Ho) and mean expected heterozygosity (He) of the 96 germplasm were 0.79 and 0.57, respectively. The mean observed heterozygosity was 0.43, 0.48, and 0.46 for the JZ, QY, and JD populations, respectively, and the mean expected heterozygosity was 0.568, 0.574, and 0.569, respectively. The QY population had the highest observed heterozygosity and expected heterozygosity, and all three populations had lower observed heterozygosity than expected heterozygosity, indicating low intra-population gene exchange.

3.3 Analysis of population genetic structure

The AMOVA revealed that much smaller variation among populations (6.5%) than within populations (19.91%+73.59%=93.50%, $p < 0.001$) (Table 5). 19.91% and 73.59% of the variation was attributed to the differences among individuals within populations and within individuals respectively. This confirmed that intra-population variation was the main source of genetic variation in the populations. The results also showed that $N_m = 3.593 > 1$, indicating that a high level of gene exchange between different populations.

Table 5
Results of Analysis of Molecular Variance of (AMOVA) of three populations of *Polygonatum cyrtoneura* Hua from seven regions

Source of variation	df	SS	MS	Est. Var.	%	P value	Nm
Among populations	2	32.995	16.497	0.203	6.50%	< 0.001	3.593
Among individuals within populations	93	328.453	3.532	0.620	19.91%	< 0.001	
Within individuals	96	220.000	2.292	2.292	73.59%	< 0.001	
Total	191	581.448		3.114	100%		
df = degree of freedom, SS = sum of squares, MS = mean squares, Est. var. = estimate of variance, % = percentage of total variation based on 999 permutations							

Fst values are used to calculate genetic differentiation among populations by means of allele frequencies, and ϕ_{st} is a measure of genetic differentiation among populations using genetic distance. The values of ϕ_{st} and Fst in the figure show that there is a certain degree of genetic differentiation between the JZ population and the QY, JD populations, while there is no obvious genetic differentiation between the QY and JD populations (Fig. 4).

3.4 Cluster analysis

3.4.1 structure analysis

The Bayesian clustering method of STRUCTURE v.2.3.4 was applied to analyze the genetic structure of 96 germplasm of three populations of *P. cyrtoneura*, and the optimal grouping K values were determined by the posterior probability LnP(D) and ΔK maximum, setting the population size K as 2 to 10. The results showed that ΔK was maximum when K = 3, as detailed in (Fig. 5), indicating that the genotypes of the 96 germplasm could be classified into three types. The three gene pools of red, blue and green were distributed in the JZ, QY and JD populations, as shown in (Fig. 6). According to the grouping results of STRUCTURE software, where red gene pools represent QY population (Qingyang, Shitai), green gene pools represent JD population (Jingde, Huangshan, Qimen), and blue gene pools represent JZ population (Jinzhai, Huoshan). The results of STRUCTURE analysis were the same as the clustering results of the study on the diversity of morphological characteristics of *P. cyrtoneura*.

3.4.2 Genetic relationship analysis of *Polygonatum cyrtoneura* Hua populations

The NJ phylogenetic tree was constructed using 96 *P. cyrtoneura* germplasm, as shown in (Fig. 7). The 96 *P. cyrtoneura* germplasm clustered into three distinct evolutionary branches, with Jinzhai and Huoshan (DH1-32) clustered into one group, Qingyang clustered into a separate group (DH32-64), other

regions such as Jingde and Shitai clustered into one group (DH64-96), and five clustered into a separate group. It can be seen that due to long-term geographical isolation, the 96 germplasm genes have produced differences, resulting in changes in plant traits, which is consistent with the analysis of the subject group's previous investigation.

4 Discussion

4.1 Method optimization

In this experiment, 14 *P. cyrtonea* germplasm from different regions were amplified using 50 pairs of SSR primers, and finally 10 pairs of SSR primers with good polymorphism were screened. Compared with the conventional polyacrylamide electrophoresis silver staining method, the fluorescence-based labeled capillary electrophoresis technique overcomes the error interference brought about in the manual operation. The fluorescence SSR can accurately record the individual fragment sizes, and the speed and accuracy of detection have been greatly improved.

4.2 Analysis of genetic diversity in different regions

Through the morphological diversity of *P. cyrtonea* plants, it was found that the plant traits of *P. cyrtonea* in different regions have produced certain differences, and then combined with the study of the habitat of *P. cyrtonea*, we divided *P. cyrtonea* into three major populations. The JZ population had tall plants with long involucre pedicels, ginger-shaped rhizomes and larger stem scars. the QY and JD populations had smaller plants with checkerboard-shaped rhizomes and smaller stem scars. Through the ecological factor analysis, the eight dominant ecological factors that contributed to the influence of *P. cyrtonea* on the suitable habitat in descending order were February precipitation, March precipitation, December precipitation, July precipitation, soil type, November precipitation, vegetation type, and slope orientation. In addition, these ecological factors are also important factors that contribute to the differences in plant morphology.

The genetic relationship and population genetic structure of 96 *P. cyrtonea* germplasm resources were analyzed. Among the 10 primer pairs, the one with the highest number of alleles was primer DH24, with an average of 7 alleles per locus. Expected heterozygosity (H_e) ranged from 0.44 to 0.77, with a mean value of 0.60. The polymorphic information content (PIC) ranged from 0.40–0.86, with a mean value of 0.72. This indicates that the 10 SSR primer pairs have highly polymorphic loci with abundant genetic variability in 96 *P. cyrtonea* germplasm resources, which could provide a basis for *P. cyrtonea* kinship identification.

The results of SSR-based molecular marker clustering showed that the 96 *P. cyrtonea* germplasm resources were divided into three populations. In terms of effective allele number (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), and genetic diversity (HS), the JZ population had more pure germplasm, the JD population had a relatively uniform allele distribution, and the QY population had the highest genetic diversity.

It can be seen that the cluster analysis of populations based on plant sexual morphological diversity is consistent with the results of cluster analysis of SSR molecular markers populations. In this paper, we investigate the genetic diversity of *P. cyrtanema* and corroborate and explain it with morphological characteristics, habitat studies and ecological factor analysis.

4.3 Analysis of the relationship between different regions

By studying the morphology of *P. cyrtanema* in different regions, they were divided into three populations according to different traits, namely JZ population, QY population and JD population. STRUCTURE cluster analysis showed that the genotypes of 96 *P. cyrtanema* germplasm resources could be classified into three types, in which the red gene pool mainly represented QY population, the green gene pool represented JD population, and the blue gene pool represented JZ population. The results of SSR molecular marker experiments were consistent with the phenotypic delineation results, and the three populations could be clustered into separate groups, with a few genes still mixed with each other.

From the NJ phylogenetic tree, it can be seen that the same communities can basically be clustered together, indicating that the clustering results can be a good representation of the species or kinship of germplasm. A relatively small proportion of the gene pool is intermixed, indicating that these materials have identical or similar parents. The classification results at the genetic level were basically consistent with the classification of various groups, in which the QY population was most closely related to the JD population, and the JZ population had genetic differentiation from the QY and JD communities. The QY and JD communities belong to the mountainous area of southern Anhui and the JZ population belongs to the Dabie Mountain area, which may lead to genetic differentiation due to different geographical environments. In the ecological factor analysis, precipitation in February and March ranked first and second among the dominant ecological factors, so it was hypothesized that precipitation during the germination and growth of *P. cyrtanema* in spring was the key ecological factor affecting its genetic differentiation. The QY population and the JD population belong to the same area of southern Anhui, and the genetic differentiation between them is low, but there have been obvious differences in plant morphology, probably because the QY population belongs to the Jiuhua Mountain system and the JD population belongs to the Huangshan Mountain system, and there are differences in vegetation and ecological environment between the two mountain systems. In addition, the transformation of a large number of wild resources into cultivation, the continuous use of rhizomes for asexual propagation may be responsible for the differences in plant morphology, and further research is needed.

5 Conclusions

In this study, we analyzed the genetic diversity and genetic structure of seven regional populations of *P. cyrtanema* using 10 pairs of SSR primers. The *P. cyrtanema* population showed high genetic diversity, with most of the variation occurring mainly within the population. The QY population has the highest genetic diversity and has great potential for use in resource conservation and breeding material selection. The JZ population in the Dabie Mountains and the QY and JD populations in the southern Anhui Mountains produced a moderate degree of genetic divergence, presumably due to long-term geographical

isolation and different natural environments. Combined with the preliminary research on plant traits, fitness zones and ecological factors, this study will provide theoretical basis and technical support for resource conservation, genetic improvement and germplasm selection and breeding of *P. cyrtanema*.

Declarations

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Author Contributions

NJ.Y. and DY.P. contributed to experimental design. ZD.W. assists with sampling of plant material. H.C. and H.L. performed the experiments and wrote the manuscript. J.X. and JY.H. contributed to modification of the manuscript. LH.X. and CC.Z. assisted with experiments and provided good advice. All authors had read and approved the final version of the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

Consent for publication All authors consent to publish.

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Figures

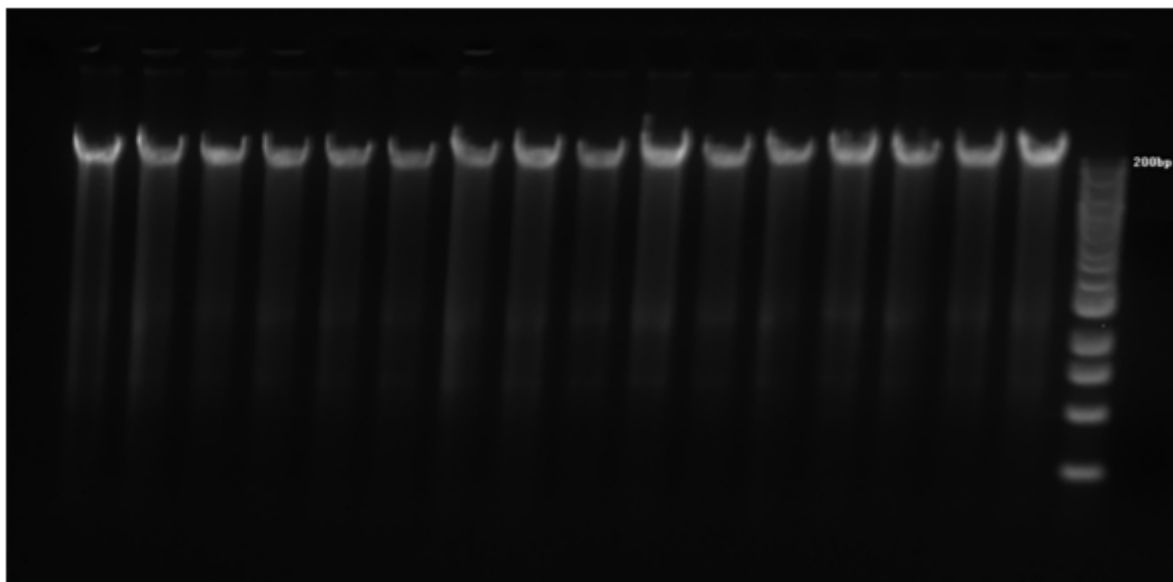


Figure 1

Electrophoresis of polymorphic fluorescent primer DH34

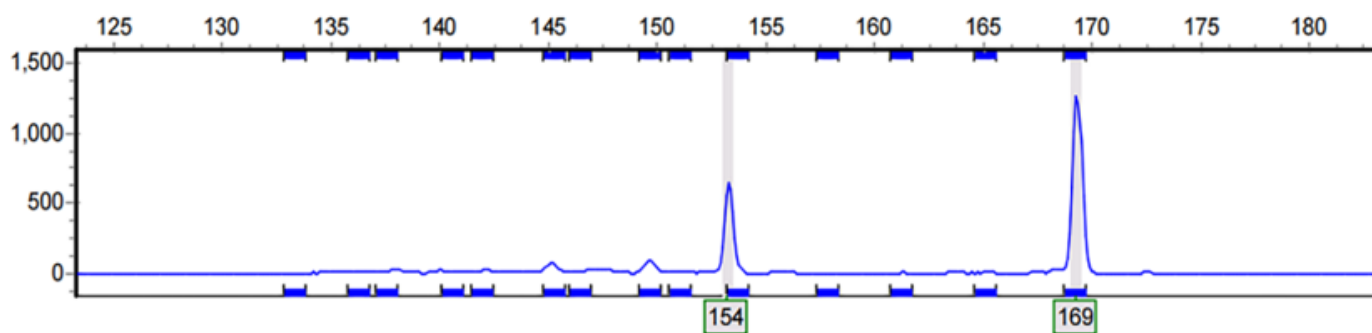


Figure 2

Polymorphic fluorescent primer DH34 capillary electrophoresis peak pattern

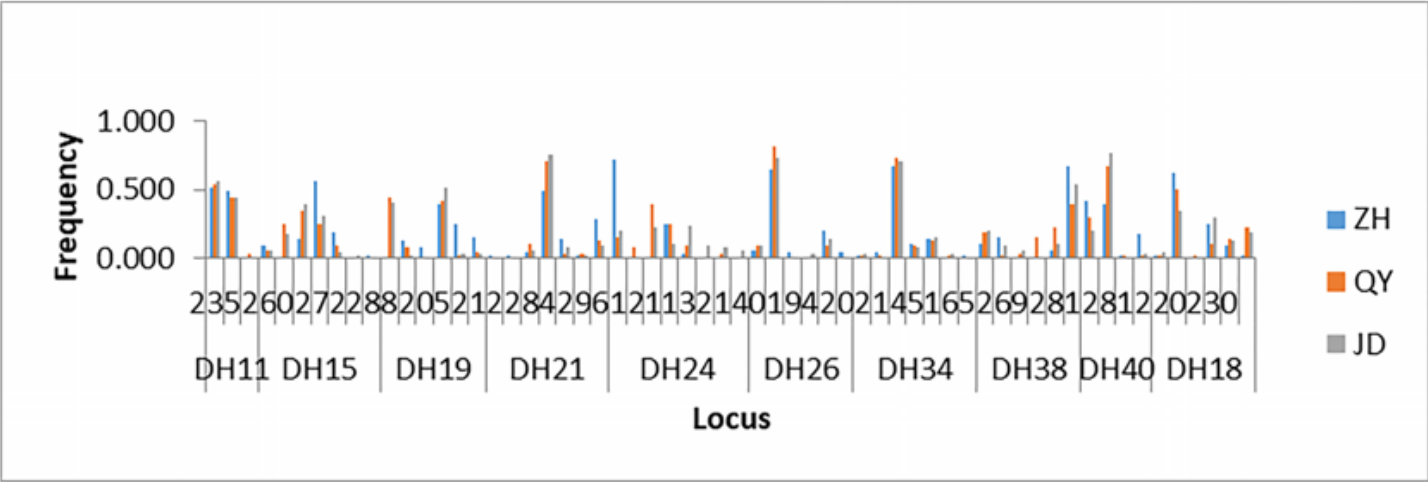


Figure 3

Gene frequency of alleles in different populations

Note: JZ: populations in Jinzhai and Huoshan areas, QY: populations in Qingyang and Shitai areas, JD: populations in Qimen, Huangshan and Jingde areas

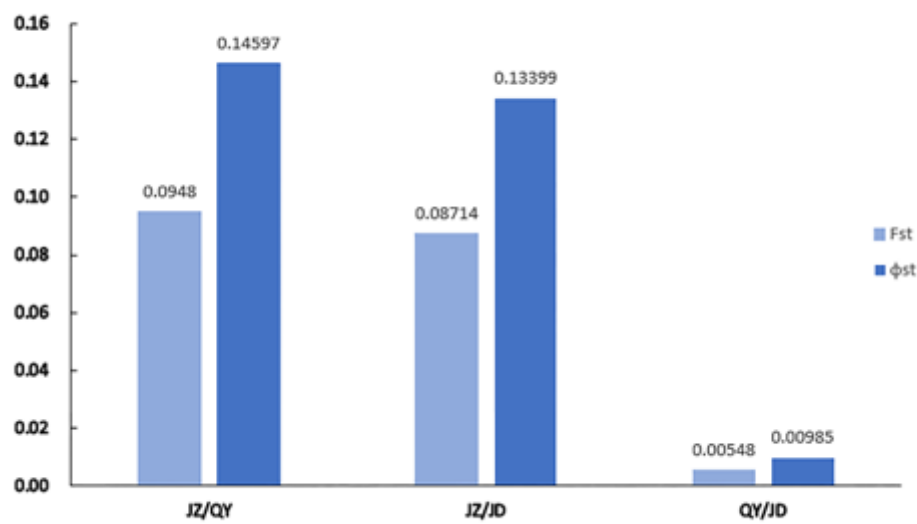


Figure 4

F_{st} ratio and ϕ_{ST} ratio among three populations

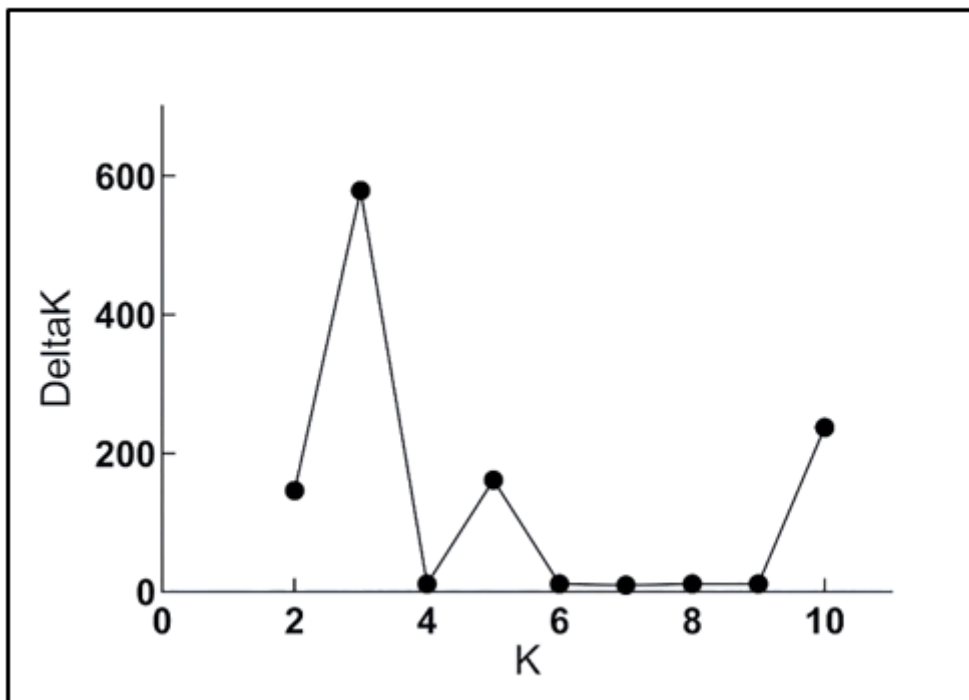


Figure 5

The optimal number of populations is 3 ($K=3$) for the ΔK value calculated by structure Harvester

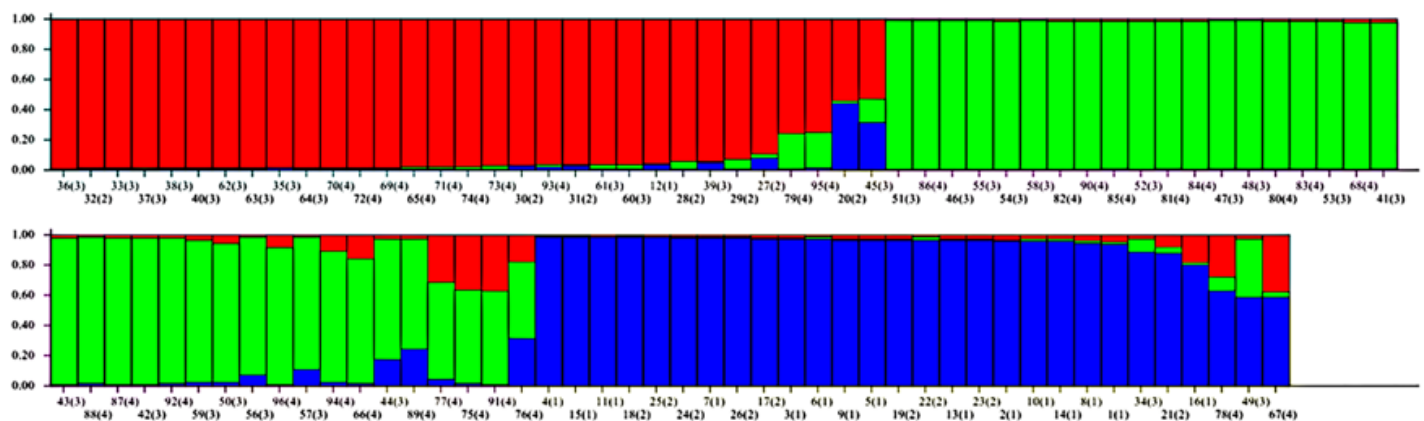


Figure 6

The relationship between the optimal number of clusters and the STUCTURE cluster analysis when $K=3$ (B)

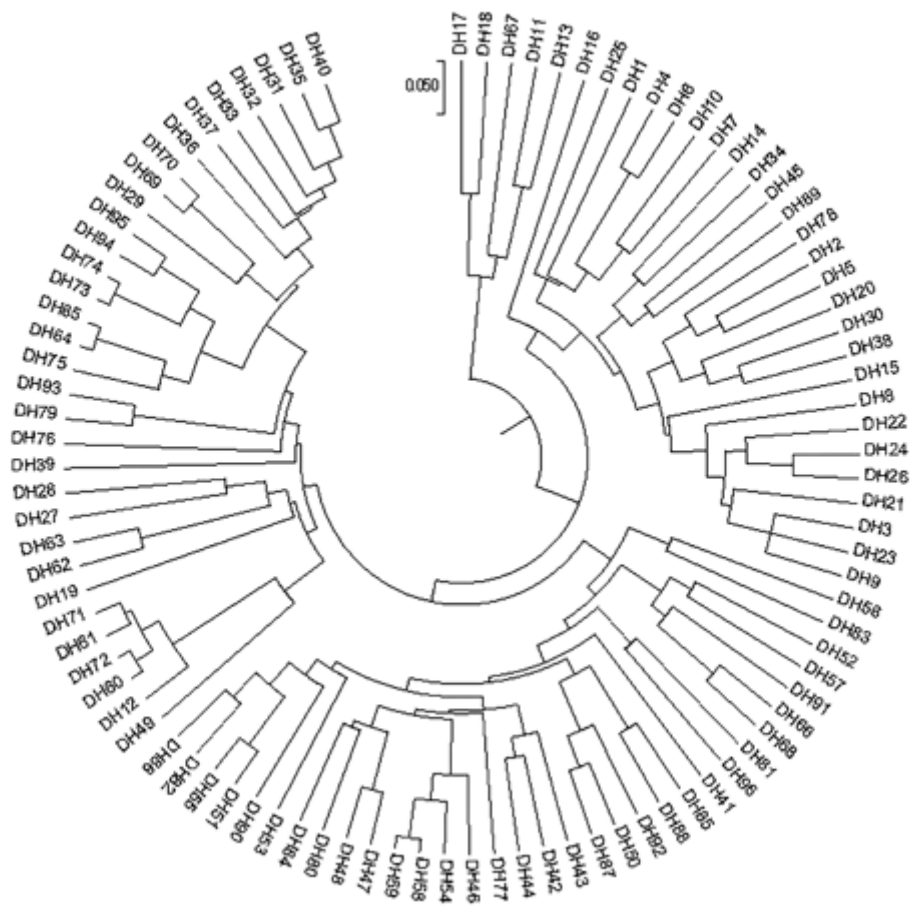


Figure 7

Construction of Nei's genetic distance distribution map of *Polygonatum cyrtonema* Hua based on 10 SSR primers