SCoT Markers Analysis of Genetic Diversity of *Polygonatum*, A Medicinal and Edible Plant

Shi dong Cao

Zhejiang Chinese Medical University

Senmiao Chen

Zhejiang Chinese Medical University

Shuyu Cao

Zhejiang Chinese Medical University

Nipi Chen

Zhejiang Chinese Medical University

Bo Jin (✉ jinbo@zcmu.edu.cn)

Zhejiang Chinese Medical University <https://orcid.org/0000-0002-7701-6102>

Research Article

Keywords: Polygonatum, SCoT, Genetic diversity, Genetic distance, Geographical distance

Posted Date: July 8th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-652340/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background: As a traditional Chinese medicine, *Polygonatum* has been demonstrated to have immunomodulatory, antibacterial, anti-inflammatory, anti-aging, anti-cancer, hypoglycemic and other pharmacological effects. However, the germplasm resources of *Polygonatum* have been destroyed in recent years and the research on its genetic diversity is extremely scarce. In this study, the genetic diversity of 28 *Polygonatum* germplasms from 11 different provinces in China was evaluated by Start codon targeted (SCoT) marker.

Results: A total of 365 bands were generated by 15 SCoT primers, of which 355 were polymorphic, with a high polymorphism of 97.3%. And the genetic similarity coefficient is between 0.59 and 0.75, indicating a high genetic diversity. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram, PCoA (Principal Coordinate Analysis) and Structure analysis have similar results in grouping *Polygonatum* germplasm and they are all divided into two populations. We found that there was a certain correlation between the genetic distance and geographical distance of *Polygonatum* germplasm. By analyzing other valid genetic diversity parameters (Na, Ne, H, I), it is clarified that *Polygonatum* has abundant alleles and abundant genetic diversity among populations.

Conclusions: This study suggests that *Polygonatum* germplasm resources from different provenances have rich genetic diversity. The SCoT molecular marker is a valuable marker system and can be used for genetic analysis of *Polygonatum* resources. This will be helpful to further study the preservation and genetic improvement of *Polygonatum* germplasm.

Introduction

Polygonatum is a traditional Chinese medicine with high medicinal and edible value. There are about 60 species in the *Polygonatum* all over the world and about 39 species are reported in China (Zhang et al. 2018). Chinese Pharmacopoeia (2015 edition) delimits Chinese medicinal *Polygonatum* from the rhizomes of three *Polygonatum* genus: *P. sibiricum* Red., *P. cyrtoneura* Hua. or *P. kingianum* Coll. et Hemsl.. The literature indicated that *Polygonatum* can enhance immunity and memory and has anti-inflammatory, anti-oxidation, anti-cancer, hypoglycemic, anti-aging effects (Khan et al. 2015; Kumar Singh and Patra 2018; Lee et al. 2016; Ma et al. 2019; Sharma et al. 2018; Wang et al. 2019; Zhang et al. 2018). Therefore, *Polygonatum* has a broad market prospect in terms of its pharmacological action and edible value. Due to the collection of *Polygonatum* is mainly based on wild resources and the supply capacity of the market is limited. Moreover, it is inevitable that there will be a mixture of different *polygonatum*, such as *Polygonatum filipes*, which has different pharmacological characteristics. However, classification within the *Polygonatum* genus through morphological identification is difficult because of variations and inter-species hybridization (Feng et al. 2020). It is necessary to assess the genetic diversity of *Polygonatum* for better understanding, conservation, and utilization of these germplasm resources.

Assessment of genetic diversity is an essential step in plant breeding programs. Molecular markers based on Polymerase chain reaction (PCR) amplification technology can directly reveal the genetic relationship between species at the DNA level and have been widely used (Feng et al. 2018; Pocza et al. 2013), such as Random Amplified Polymorphic DNA (RAPD) (Cui et al. 2017), Inter-simple sequence repeat (ISSR) (Kumar et al. 2016), Sequence related amplified polymorphism (SRAP) (Zhao et al. 2020). However, the complexity, stability and ability of generating genetic information of molecular marker technology are different, and each has its advantages and disadvantages. Start codon targeted (SCoT) marker is designed by Collard and Mackill based on the short conserved sequences flanking the start codon (ATG) in plant genes (Collard and Mackill 2008). SCoT markers are related to functional genes, easy to operate, universal primers, higher annealing temperature (50°C), longer primers (18-mer), higher polymorphism and distinguishability (Golkar and Mokhtari 2018; Jalilian et al. 2018; Safari et al. 2019; Xiong et al. 2011; Yang et al. 2019). SCoT markers have been successfully applied to research on genetic diversity of a variety of crops, flowers, etc. (Tyagi et al. 2020), including *Polygonatum* (Feng et al. 2020; Jiao et al. 2018).

In this study, SCoT markers were used to evaluate the genetic diversity of 28 *Polygonatum* germplasms and the genetic relationship between the provenances of *Polygonatum* from different sources is discussed. This provides references for the selection of *Polygonatum* germplasm resources, breed selection, production of Chinese medicinal materials, and provides a theoretical basis for the protection of *Polygonatum* resources.

Materials And Methods

Plant materials

In this study, a total of 28 *Polygonatum* germplasm resources from 11 different provinces in China were evaluated, covering most of the major distribution areas in China. Table 1 lists the relevant information of the sample germplasm. Fig. 1 shows the 11 different provinces of origin. The voucher specimens of plant materials have been deposited in the Laboratory of Molecular Biology of Zhejiang Chinese Medical University (No.: BJ-20160301-001 to NO.BJ20160301-050).

DNA extraction

Plant DNA was extracted from the fresh leaves by using modified cetyltrimethylammonium bromide (CTAB) method (Abarshi et al. 2010; Murray and Thompson 1980). 1% agarose gel electrophoresis was used to evaluate the quality and integrity of the extracted genomic DNA, and then stored at -20 °C for subsequent PCR amplification.

Table 1. *Polygonatum* germplasm resources from different geographical regions of China used in this study

Serial number	Polygonatum Accessions	Origin	Abbreviation
1	<i>Polygonatum cyrtonema</i> Hua.	Xiaoshan, Hangzhou, Zhejiang	PcH1
2	<i>Polygonatum sibiricum</i> .	Hangping, Pujiang, Zhejiang	Ps2
3	<i>Polygonatum cyrtonema</i> Hua.	Mountain, Jiangshan, Zhejiang	PcH3
4	<i>Polygonatum cyrtonema</i> Hua.	Yuyao, Ningbo, Zhejiang	PcH4
5	<i>Polygonatum sibiricum</i> .	Yuyao, Ningbo, Zhejiang	Ps5
6	<i>Polygonatum filipes</i> .	Ninghai, Ningbo, Zhejiang	Pf6
7	<i>Polygonatum sibiricum</i> .	Ninghai, Ningbo, Zhejiang	Ps7
8	<i>Polygonatum cyrtonema</i> Hua.	Tonglu, Hangzhou, Zhejiang	PcH8
9	<i>Polygonatum cyrtonema</i> Hua.	Cili, Zhangjiajie, Hunan	PcH9
10	<i>Polygonatum sibiricum</i> .	Cili, Zhangjiajie, Hunan	Ps10
11	<i>Polygonatum sibiricum</i> .	Huitong, Huaihua, Hunan	Ps11
12	<i>Polygonatum sibiricum</i> .	Xinhua, Loudi, Hunan	Ps12
13	<i>Polygonatum sibiricum</i> Red.	Xian'an, Xianning, Hubei	PsR13
14	<i>Polygonatum cyrtonema</i> Hua.	Xian'an, Xianning, Hubei	PcH14
15	<i>Polygonatum sibiricum</i> .	Chongyang, Xianning, Hubei	Ps15
16	<i>Polygonatum sibiricum</i> .	Yangxin, Huangshi, Hubei	Ps16
17	<i>Polygonatum sibiricum</i> .	Li, Longnan, Gansu	Ps17
18	<i>Polygonatum sibiricum</i> .	Xianghe, Shangnan, Shaanxi	Ps18
19	<i>Polygonatum sibiricum</i> .	Shimian, Ya'an, Sichuan	Ps19
20	<i>Polygonatum sibiricum</i> Red.	Shimian, Ya'an, Sichuan	PsR20
21	<i>Polygonatum sibiricum</i> Red.	Yimen, Yuxi, Yunnan	PsR21
22	<i>Polygonatum kingianum</i> Coll.et Hemsl. <i>Polygonatum sibiricum</i> .	Mengzi, Honghe, Yunnan	PkCH22
23	<i>Polygonatum sibiricum</i> Red.	Libo, Qiannan, Guizhou	Ps23
24	<i>Polygonatum sibiricum</i> .	Chishui, Zunyi, Guizhou	PsR24
25	<i>Polygonatum cyrtonema</i> Hua.	Baiwanyao, Xingyi, Guizhou	Ps25
26	<i>Polygonatum cyrtonema</i> Hua.	Yizhou, Hechi, Guangxi	PcH26
27	<i>Polygonatum sibiricum</i> .	Changting, Longyan, Fujian	PcH27
28		Yuanzhou, Yichun, Jiangxi	Ps28

Polymerase chain reaction and agarose gel electrophoresis

Based on the analysis of two randomly selected germplasms, among 36 SCoT primers (Shanghai Shenggong Bioengineering Service Co., Ltd.) (Collard and Mackill 2008), primers with clear and rich polymorphic bands were selected to evaluate the genetic diversity of *Polygonatum* germplasm. The total volume of the PCR amplification system was 20 µl, 0.8 µl of primers (10 µM), 10 µl of 2 × Es Taq Master Mix (Beijing Kangwei Century Biotechnology Co., Ltd.), 1 µl of genomic DNA template, and 8.2 µl ddH₂O. S1000™ Thermal Cycler PCR (Shenzhen Yudeli Biotechnology Co., Ltd.) was used for amplification. The setup procedure was: an initial step at 95 °C for 4 min, followed by 36 cycles of 95 °C for 50 s, 72 °C for 2 min, and a 5 min final extension at 72 °C. 10 µl PCR amplification products were analyzed by electrophoresis in 1.5% agarose gel containing 5 mg/ml ethidium bromide and then observed and photographed under the ZF-90 dark box ultraviolet transmittance meter (Shanghai Gucun Electro-Optic instrument Factory).

Data analysis

In the products amplified by SCoT primers, only the clear and repeatable DNA bands were marked as (1), and the non-existence were marked as (0) and then a binary matrix was constructed. Cluster analysis was performed using NTSYS-pc version 2.10e software (Powell et al. 1996) and the Principal coordinate analysis (PCoA) two-dimensional distribution map was obtained. The unweighted Pair Group Method with Arithmetic Mean (UPGMA) and the dissimilarity index in Jaccard's option were used to construct a dendrogram (Zhao et al. 2020). Nei's genetic identity (H) (Nei 1972). Effective number of alleles (Ne), Shannon's information index (I) and other genetic diversity indexes were obtained by POPGENE software.1.32 (Yeh et al. 1997). In addition, the formula $PIC = 2f_i(1-f_i)$ was used to calculate the polymorphic information content (PIC). Where " f_i " is the frequency of the amplified allele (band presence) and " $1-f_i$ " is the frequency of the null allele (Powell et al. 1996; Zargar et al. 2016). In addition, the genetic structure of the population was analyzed by STRUCTURE software ver.2.3.4. In order to determine the best K value of the population, the K value was preset from 1 to 15 and each K value was run five times independently. The burn-in period parameter was configured to 100,000 and the number of Markov Chain Monte Carlo (MCMC) (Bayesian statistics) replications (simulations) after burn-in was set over 1,000,000 (Karandikar 2006; Li et al. 2020). An online tool, Structure Harvester, is then used to process results of STRUCTURE analysis, using the delta K (ΔK) method to find the best K value (Earl and vonHoldt 2012).

Results

SCoT analysis

From 36 SCoT primers, 15 primers that can generate reproducible and high-resolution polymorphic fragments were selected for sample research (Table 2). A total of 365 credible bands were obtained, of which 355 were polymorphic, and the percentage of polymorphism was 97.2%. The bands amplified by each primer range from 19 (SCoT 11) to 32 (SCoT 7), with an average of 24.3 and the number of polymorphic bands ranged from 18 (SCoT 11) to 32 (SCoT 7), with an average of 23.7. The percentage of polymorphism ranged from 87.5% (SCoT 14) to 100% (SCoT 28), each primer has 18-32 polymorphic bands, and SCoT 7 produced the most polymorphic bands (PB = 32). PIC values ranged from 0.307 (SCoT 14) to 0.426 (SCoT 33), with an average of 0.379 (Table 2). The amplification results showed that SCoT markers could be used to detect the high polymorphism of *Polygonatum* germplasm.

Table 2. Sequence and polymorphism information of the 15 SCOT primers selected in this study

Primer code	Primer sequence (5'-3')	No. of amplified bands	No. of polymorphic bands (PB)	Polymorphic (%)	Polymorphic information content (PIC)
SCoT1	CAACAATGGCTACCACCA	31	30	96.8	0.384
SCoT 2	CAACAATGGCTACCACCC	22	22	100.0	0.413
SCoT 6	CAACAATGGCTACCACGC	23	22	95.7	0.386
SCoT 7	CAACAATGGCTACCACGG	32	32	100.0	0.380
SCoT 11	AAGCAATGGCTACCACCA	19	18	94.7	0.363
SCoT 13	ACGACATGGCGACCATCG	25	24	96.0	0.352
SCoT 14	ACGACATGGCGACCACGC	24	21	87.5	0.307
SCoT 15	ACGACATGGCGACCGCGA	27	27	100.0	0.409
SCoT 17	ACCATGGCTACCACCGAG	21	21	100.0	0.341
SCoT 20	ACCATGGCTACCACCGCG	22	21	95.5	0.383
SCoT 22	AACCATGGCTACCACCAC	21	20	95.2	0.341
SCoT 28	CCATGGCTACCACCGCCA	20	20	100.0	0.410
SCoT 29	CCATGGCTACCACCGGCC	25	25	100.0	0.406
SCoT 31	CCATGGCTACCACCGCCT	28	27	96.4	0.377
SCoT 33	CCATGGCTACCACCGCAG	25	25	100.0	0.426
Mean	-	24.3	23.7	97.2	0.379
Total	-	365	355	-	-

Population genetic diversity

According to the provincial sources, 28 samples were divided into eleven populations, representing different provinces, table 3 shows their genetic diversity index. The values of Observed number of alleles (N_a), Effective number of alleles (N_e), Nei's gene diversity (H) and Shannon's Information index (I) in Zhejiang Province were the highest, $N_a = 1.8630$, $N_e = 1.4561$, $H = 0.2796$, $I = 0.4275$, and the standard deviations were 0.3443, 0.3129, 0.1601, 0.2194, respectively. The corresponding parameters of genetic diversity in Sichuan Province were the lowest, $N_a = 1.3315$, $N_e = 1.2344$, $H = 0.1373$, $I = 0.2005$, and the standard deviations were 0.4714, 0.3333, 0.1953 and 0.2851, respectively. Among the 15 SCoT markers (Table 4), the spans of N_e , H , I values are 1.4057-1.7173, 0.2546-0.3947, 0.3959-0.5737, respectively and the standard deviations are 0.2466-0.3835, 0.1104-0.1834, 0.1302-0.2381 respectively.

According to Nei's genetic identity and genetic distance, the highest genetic identity (0.9216) and the minimum genetic distance (0.0816) was recorded between Zhejiang province and Hunan province, while the lowest identity (0.5370) and the maximum genetic distance (0.6218) was recorded between Gansu province and Jiangxi province (Table 5). These results indicate that *Polygonatum* has high genetic diversity and SCoT markers can significantly distinguish the genetic differences of *Polygonatum* germplasm.

Table 3. Genetic diversity parameters for different provinces of *Polygonatum*

Province	Sample size	Na	Ne	H	I
Zhejiang	8	1.8630(0.3443)	1.4561(0.3129)	0.2796(0.1601)	0.4275(0.2194)
Hunan	4	1.6712(0.4704)	1.4084(0.3634)	0.2416(0.1915)	0.3621(0.2732)
Hubei	4	1.7315(0.4438)	1.4552(0.3696)	0.2260(0.1883)	0.3974(0.2648)
Yunnan	2	1.3781(0.4856)	1.2673(0.3434)	0.1566(0.2011)	0.2286(0.2936)
Guizhou	3	1.5973(0.4911)	1.3822(0.3723)	0.2244(0.1979)	0.3340(0.2854)
Sichuan	2	1.3315(0.4714)	1.2344(0.3333)	0.1373(0.1953)	0.2005(0.2851)
Gansu	1	1.0000(0.0000)	1.0000(0.0000)	0.0000(0.0000)	0.0000(0.0000)
Shanxi	1	1.0000(0.0000)	1.0000(0.0000)	0.0000(0.0000)	0.0000(0.0000)
Jiangxi	1	1.0000(0.0000)	1.0000(0.0000)	0.0000(0.0000)	0.0000(0.0000)
Fujian	1	1.0000(0.0000)	1.0000(0.0000)	0.0000(0.0000)	0.0000(0.0000)
Guangxi	1	1.0000(0.0000)	1.0000(0.0000)	0.0000(0.0000)	0.0000(0.0000)
Mean	-	1.5954	1.3673	0.2109	0.3250

Note: Standard deviations are in parentheses, *Na* Observed number of alleles, *Ne* Effective number of alleles, *H* Nei's gene diversity, *I* Shannon's Information index

Table 4. Analysis of genetic diversity of *Polygonatum* by SCoT markers

Maker	Ne	H	I
SCoT1	1.5237(0.2616)	0.3232(0.1255)	0.4936(0.1562)
SCoT2	1.6093(0.2975)	0.3562(0.1287)	0.5321(0.1535)
SCoT6	1.4946(0.2466)	0.3562(0.1701)	0.4811(0.1539)
SCoT7	1.4756(0.2683)	0.2992(0.1333)	0.4629(0.1705)
SCoT11	1.5820(0.3619)	0.3304(0.1752)	0.4893(0.2285)
SCoT13	1.5224(0.3298)	0.3111(0.1567)	0.4705(0.2055)
SCoT14	1.4057(0.3093)	0.2546(0.1638)	0.3959(0.2246)
SCoT15	1.5489(0.2575)	0.3357(0.1189)	0.5095(0.1460)
SCoT17	1.4914(0.3183)	0.3004(0.1450)	0.4639(0.1770)
SCoT20	1.5330(0.2892)	0.3238(0.1356)	0.4911(0.1749)
SCoT22	1.5803(0.3835)	0.3254(0.1834)	0.4813(0.2381)
SCoT28	1.7173(0.3115)	0.3947(0.1352)	0.5737(0.1617)
SCoT29	1.5684(0.2767)	0.3416(0.1250)	0.5156(0.1520)
SCoT31	1.5249(0.2955)	0.3195(0.1344)	0.4871(0.1691)
SCoT33	1.5971(0.2618)	0.3567(0.1104)	0.5353(0.1302)
Mean	1.4396	0.3286	0.4922

Note: Standard deviations are in parentheses, *Na* Observed number of alleles, *Ne* Effective number of alleles, *H* Nei's gene diversity, *I* Shannon's Information index

Table 5. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among eleven provinces of *Polygonatum*

POP ID	Zhejiang	Hunan	Hubei	Guizhou	Gansu	Shanxi	Jiangxi	Yunnan	Guangxi	Fujian	Sichuan
Zhejiang	****	0.9216	0.9185	0.9266	0.6892	0.7179	0.7809	0.8814	0.7765	0.7805	0.8947
Hunan	0.0816	****	0.8809	0.8679	0.6637	0.6797	0.7769	0.8501	0.7568	0.7668	0.8451
Hubei	0.0850	0.1268	****	0.8891	0.7064	0.7244	0.7365	0.8079	0.7524	0.7606	0.8381
Guizhou	0.0762	0.1416	0.1175	****	0.6881	0.6937	0.7295	0.8454	0.7334	0.7663	0.8644
Gansu	0.3722	0.4100	0.3475	0.3738	****	0.5973	0.5370	0.6538	0.5753	0.5726	0.6291
Shanxi	0.3315	0.3861	0.3224	0.3657	0.5154	****	0.6164	0.6364	0.6000	0.6137	0.6691
Jiangxi	0.2474	0.2524	0.3058	0.3154	0.6218	0.4838	****	0.7151	0.6822	0.6411	0.7126
Yunnan	0.1263	0.1624	0.2133	0.1679	0.4250	0.4520	0.3354	****	0.7238	0.7409	0.8369
Guangxi	0.2529	0.2786	0.2845	0.3101	0.5528	0.5108	0.3824	0.3232	****	0.7288	0.7303
Fujian	0.2478	0.2655	0.2736	0.2661	0.5576	0.4883	0.4446	0.2999	0.3164	****	0.7339
Sichuan	0.1112	0.1683	0.1767	0.1457	0.4635	0.4018	0.3389	0.178	0.3143	0.3093	****

Cluster analysis and Population structure

Cluster analysis of 28 germplasm materials was carried out by UPGMA program and a tree diagram was generated. On the whole, it is divided into two groups: Group I and Group II, when the similarity index is 0.606, these germplasm materials can be clustered into four subgroups (Fig. 2). Two-dimensional PCoA analysis of SCoT markers amplification products showed that *Polygonatum* germplasm was mainly divided into two groups, which was similar to the results shown by UPGMA dendrogram (Fig. 3). In addition, the correlation analysis also shows that the similarity matrix fits well and the similarity coefficient is 0.7335, indicating that the two analysis methods (PCoA and UPGMA) have obtained comparable results (Fig. 4).

STRUCTURE software ver.2.3.4 was used to estimate the population structure of 28 accessions based on fragments obtained from 15 SCoT primers. Structure Harvester was used to estimate the best number of populations using the ΔK method (Earl and vonHoldt 2012). The highest value for ΔK was obtained at $K = 2$ ($\Delta K = 137.01$); indicating the entire germplasm could be divided into two groups (Fig. 5). The results indicated that STRUCTURE analysis, PCoA analysis and UPGMA analysis had similar results and the same germplasm was divided into two populations. In Group I, four germplasms belong to Zhejiang, two germplasms belong to Hunan, three germplasms belong to Hubei, and the rest belong to Guizhou, Shaanxi, Gansu. In Group II, Zhejiang contains four germplasms, Hunan, Yunnan, Guizhou, Sichuan contain two germplasms respectively and Hubei, Guangxi, Jiangxi, Fujian each have one.

Discussion

Studying the genetic diversity of plants is of great significance to plant breeding and genetic protection. Genetic evaluation conduces to broaden the genetic basis of varieties and prevent gene insertion or deletion (Mulpuri et al. 2013; Talebi et al. 2008). DNA molecular marker technology with higher polymorphism and higher accuracy provides a simple and effective method for studying the genetic diversity of *Polygonatum* germplasm (Yu et al. 2017). Unlike RAPD, Inter-simple sequence repeat (ISSR) and other similar markers, SCoT markers are related to functional genes and corresponding traits and have better marker resolution, so they are suitable for marker-assisted breeding (Collard and Mackill 2008; Feng et al. 2016; Mulpuri et al. 2013). In recent years, SCoT markers have been widely used to reveal the genetic diversity of many plants, such as *Green Tea* (Xu et al. 2019), *Bletilla striata* (Guo et al. 2018), *Capsicum annuum* (Igwe et al. 2019), *Miscanthus lutarioriparius* (Yang et al. 2019), etc. Even though there have been related reports on *Polygonatum* germplasm (Jiao et al. 2018), based on SCoT markers, few primers are used and the analysis method is single, the data may lack validity.

In this study, the percentage of polymorphic bands (PPB) amplified by 15 SCoT primers was 97.2%, which was higher than that in *Mango* (PPB = 76.19%) (Luo et al. 2010), *Switchgrass* (PPB = 90%) (Zhang et al. 2015). According to PIC value (PIC = 0.379), SCoT markers are highly polymorphic and informative. In addition, the high polymorphism of SCoT markers also indicates that there is considerable genetic diversity among tested *Polygonatum* varieties.

The average value of population genetic diversity is 0.21, which is higher than the previous analysis in *Polygonatum* ($H = 0.1943$, $H = 0.1279$) (Feng et al. 2020), indicating that SCoT markers are more superior than EST-SSR markers and SRAP markers. However, in previous reports that also used SCoT markers, H values were found to be lower than in *Capsicum annuum* ($H = 0.5936$) and *Miscanthus lutarioriparius* ($H = 0.32$), which may be due to different germplasm or the number of individuals in the population (Igwe et al. 2019; Yang et al. 2019). The average value of genetic diversity in SCoT marker is 0.3286, which also indicates that SCoT marker is highly polymorphic. But it is lower than in *Vigna unguiculata* L (Igwe et al. 2017), this may be due to the different sample germplasm used by both parties. According to Nei's genetic identity and genetic distance, it can be found that the germplasm of Hunan and Zhejiang are highly correlated. This indicates that there may be frequent gene flow among *Polygonatum* species between the two provinces in recent years, and similar results have been found in the study of commercial and wild pear species (Jalilian et al. 2018).

According to Cluster analysis, *Polygonatum* germplasm was divided into two populations by UPGMA dendrogram. PCoA analysis and population structure analysis also proved this result. Most studies using SCoT markers have used similar methods and have similar results (Golkar and Mokhtari 2018; Xu et al. 2019). When the similarity coefficient is 0.606, the two populations can be divided into four subgroups. From the map (Fig. 1), we can find that Zhejiang Province, Hubei Province, Hunan Province are geographically close, and subgroup I mainly clusters most of the germplasm in these three provinces together. Subgroup II contains four different provinces, and also have close geographical locations. Subgroup III contains only one sample from the mountainous area, and this may be due to the geographic blockage of information communication. Similar results were found in *Miscanthus lutarioriparius* (Yang et al. 2019). Subgroup IV clusters several provinces with close geographical location, such as Yunnan, Guizhou, Sichuan, and Jiangsu, Zhejiang, Fujian. *Polygonatum* germplasm in the same province will basically be grouped into the same category, like Hunan and Sichuan. However, we found that *Polygonatum* germplasm in the same province may be divided into different populations, such as Zhejiang, Hubei, Hunan, Guizhou. This shows that the genetic communication of *Polygonatum* in some areas will still be limited, although they are not far apart. We also found that some germplasms from Fujian and Sichuan or Gansu and Zhejiang, which are far away from each other, have also been grouped together. This may be due to the convenient transportation, the rapid development of network information, the frequent intensification of human activities and the increase of information exchange among species. In addition, the dendrogram shows that *Polygonatum* of the same species is mainly clustered into different subgroups. This may be caused by the geographical distance of these species. Among them, there are great similarities between *Polygonatum sibiricum*. and *Polygonatum cyrtoneura* Hua., and between *Polygonatum sibiricum*. and *Polygonatum sibiricum* Red.. This may be caused by interspecific hybridization between *Polygonatum*. *Polygonatum kingianum* Coll.et Hemsl. was clustered together with *Polygonatum sibiricum*. and *Polygonatum cyrtoneura* Hua. This result is similar to the previous report by Jiao et al (Jiao et al. 2018). In summary, *Polygonatum* germplasm has higher genetic diversity. The genetic distance and geographical distance of *Polygonatum* germplasm have a certain degree of correlation. This provides an important reference value for the construction of DNA fingerprint, population structure analysis, germplasm preservation and breeding of *Polygonatum*. For example, *Polygonatum* within the same province or between neighboring provinces should avoid gene interflow as much as possible, and *Polygonatum* with far geographical distance can be used as parents for hybridization.

Conclusion

In this study, we evaluated 28 *Polygonatum* germplasms from 11 different provinces in China using SCoT molecular markers. The results showed that *Polygonatum* germplasm has higher genetic diversity. In addition, UPGMA cluster analysis and STRUCTURE analysis showed that the genetic distance of *Polygonatum* germplasm was correlated with geographical distribution to some extent. Among them, geographical location, human activities, climate, temperature, and other conditions will affect the genetic communication of *Polygonatum* germplasm. The number of samples is often closely related to the study of genetic diversity. To further understand the genetic diversity of *Polygonatum*, it is necessary to use other efficient molecular marker techniques and use more widely *Polygonatum* samples for in-depth analysis.

Declarations

Funding

This work was supported by the Natural Science Foundation of Zhejiang Province, China (LY17H280007).

Competing interests

The authors declare that they have no conflict of interest.

Availability of data and material

The data used to support the findings of this study are available from the corresponding author upon request.

Code availability

Not applicable.

Authors' contributions

Bo Jin and Nipi Chen conceived and designed the study. Shidong Cao, Senmiao Chen and Shuyu Cao performed the experiments. Shidong Cao wrote the paper. Shidong Cao reviewed and edited the manuscript. All authors read and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publication.

Acknowledgements

We are very grateful to the teachers and reviewers for their valuable help in completing this article.

References

1. Abarshi MM, Mohammed IU, Wasswa P, Hillocks RJ, Holt J, Legg JP, Seal SE, Maruthi MN (2010) Optimization of diagnostic RT-PCR protocols and sampling procedures for the reliable and cost-effective detection of Cassava brown streak virus. *Journal of virological methods* 163:353-359
2. Collard BCY, Mackill DJ (2008) Start Codon Targeted (SCoT) Polymorphism: A Simple, Novel DNA Marker Technique for Generating Gene-Targeted Markers in Plants. *Plant Molecular Biology Reporter* 27:86. <https://doi:10.1007/s11105-008-0060-5>
3. Cui C, Li Y, Liu Y, Li X, Luo S, Zhang Z, Wu R, Liang G, Sun J, Peng J, Tian P (2017) Determination of genetic diversity among Saccharina germplasm using ISSR and RAPD markers. *Comptes rendus biologies* 340:76-86
4. Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4:359-361. <https://doi:10.1007/s12686-011-9548-7>
5. Feng S, He R, Jiang M, Lu J, Shen X, Liu J, Wang Z, Wang H (2016) Genetic diversity and relationships of medicinal *Chrysanthemum morifolium* revealed by start codon targeted (SCoT) markers. *Scientia Horticulturae* 201:118-123
6. Feng S, Zhu Y, Yu C, Jiao K, Jiang M, Lu J, Shen C, Ying Q, Wang H (2018) Development of Species-Specific SCAR Markers, Based on a SCoT Analysis, to Authenticate *Physalis* (Solanaceae) Species. *Frontiers in genetics* 9:192
7. Feng T, Jia Q, Meng X, Chen X, Wang F, Chai W, Liang Z (2020) Evaluation of genetic diversity and construction of DNA fingerprinting in *Polygonatum* Mill. based on EST-SSR and SRAP molecular markers. *3 Biotech* 10:322. <https://doi:10.1007/s13205-020-02316-z>
8. Golkar P, Mokhtari N (2018) Molecular diversity assessment of a world collection of safflower genotypes by SRAP and SCoT molecular markers. *Physiology and molecular biology of plants : an international journal of functional plant biology* 24:1261-1271. <https://doi:10.1007/s12298-018-0545-0>
9. Guo Y, Zhai L, Long H, Chen N, Gao C, Ding Z, Jin B (2018) Genetic diversity of *Bletilla striata* assessed by SCoT and IRAP markers. *Hereditas* 155:35. <https://doi:10.1186/s41065-018-0074-4>
10. Igwe DO, Afiukwa CA, Acquah G, Ude GN (2019) Genetic diversity and structure of *Capsicum annuum* as revealed by start codon targeted and directed amplified minisatellite DNA markers. *Hereditas* 156:32. <https://doi:10.1186/s41065-019-0108-6>

11. Igwe DO, Afiukwa CA, Ubi BE, Ogbu KI, Ojuederie OB, Ude GN (2017) Assessment of genetic diversity in *Vigna unguiculata* L. (Walp) accessions using inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) polymorphic markers. *BMC genetics* 18:98. <https://doi.org/10.1186/s12863-017-0567-6>
12. Jalilian H, Zarei A, Erfani-Moghadam J (2018) Phylogeny relationship among commercial and wild pear species based on morphological characteristics and SCoT molecular markers. *Scientia Horticulturae* 235:323-333
13. Jiao J, Jia X, Liu P, Zhang Q, Liu F, Ma C, Xi P, Liang Z (2018) Species identification of polygonati rhizoma in China by both morphological and molecular marker methods. *Comptes rendus biologies* 341:102-110
14. Karandikar RL (2006) On the Markov Chain Monte Carlo (MCMC) method. *Sadhana* 31:81-104. <https://doi.org/10.1007/BF02719775>
15. Khan H, Saeed M, Rauf A, Khan MA, Muhammad N (2015) Antimicrobial and inhibition on heat-induced protein denaturation of constituents isolated from *Polygonatum verticillatum* rhizomes. *Natural product research* 29:2160-2163
16. Kumar H, Priya P, Singh N, Kumar M, Choudhary BK, Kumar L, Singh IS, Kumar N (2016) RAPD and ISSR Marker-Based Comparative Evaluation of Genetic Diversity Among Indian Germplasms of *Euryale ferox*: an Aquatic Food Plant. *Applied biochemistry and biotechnology* 180:1345-1360. <https://doi.org/10.1007/s12010-016-2171-z>
17. Kumar Singh S, Patra A (2018) Evaluation of phenolic composition, antioxidant, anti-inflammatory and anticancer activities of *Polygonatum verticillatum* (L.). *Journal of integrative medicine* 16:273-282
18. Lee JE, Kim EJ, Kim MH, Hong J, Yang WM (2016) *Polygonatum stenophyllum* improves menopausal obesity via regulation of lipolysis-related enzymes. *Journal of natural medicines* 70:789-796. <https://doi.org/10.1007/s11418-016-1018-9>
19. Li ST, Ramakrishnan M, Vinod KK, Kalendar R, Yrjala K, Zhou MB (2020) Development and Deployment of High-Throughput Retrotransposon-Based Markers Reveal Genetic Diversity and Population Structure of Asian Bamboo. *Forests* 11. <https://doi.org/10.3390/f11010031>
20. Luo C, He X-H, Chen H, Ou S-J, Gao M-P (2010) Analysis of diversity and relationships among mango cultivars using Start Codon Targeted (SCoT) markers. *Biochemical Systematics and Ecology* 38:1176-1184
21. Ma YL, Zhang YS, Zhang F, Zhang YY, Thakur K, Zhang JG, Wei ZJ (2019) Methyl protodioscin from *Polygonatum sibiricum* inhibits cervical cancer through cell cycle arrest and apoptosis induction. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 132:110655
22. Mulpuri S, Muddanuru T, Francis G (2013) Start codon targeted (SCoT) polymorphism in toxic and non-toxic accessions of *Jatropha curcas* L. and development of a codominant SCAR marker. *Plant science : an international journal of experimental plant biology* 207:117-127
23. Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic acids research* 8:4321-4325. <https://doi.org/10.1093/nar/8.19.4321>
24. Nei M (1972) Genetic Distance between Populations. *The American Naturalist* 106:283-292. <https://doi.org/10.1086/282771>
25. Poczai P, Varga I, Laos M, Cseh A, Bell N, Valkonen JP, Hyvönen J (2013) Advances in plant gene-targeted and functional markers: a review. *Plant methods* 9:6. <https://doi.org/10.1186/1746-4811-9-6>
26. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* 2:225-238. <https://doi.org/10.1007/BF00564200>
27. Safari H, Zebajadi A, Kahrizi D, Jafari AA (2019) The study of inter-specific relationships of *Bromus* genus based on SCoT and ISSR molecular markers. *Molecular biology reports* 46:5209-5223. <https://doi.org/10.1007/s11033-019-04978-2>
28. Sharma S, Patial V, Singh D, Sharma U, Kumar D (2018) Antimicrobial Homoisoflavonoids from the Rhizomes of *Polygonatum verticillatum*. *Chemistry & biodiversity* 15:e1800430. <https://doi.org/10.1002/cbdv.201800430>
29. Talebi R, Fayaz F, Mardi M, Pirsyedi SM, Naji M (2008) Genetic Relationships Among Chickpea (*Cicer Arietinum* L.) Elite Lines Based on Rapd and Agronomic Markers. *International Journal of Agriculture & Biology* 10:301-305
30. Tyagi R, Sharma V, Sureja AK, Das Munshi A, Arya L, Saha D, Verma M (2020) Genetic diversity and population structure detection in sponge gourd (*Luffa cylindrica*) using ISSR, SCoT and morphological markers. *Physiology and molecular biology of plants : an international journal of functional plant biology* 26:119-131. <https://doi.org/10.1007/s12298-019-00723-y>
31. Wang C et al. (2019) Transcriptome analysis of *Polygonatum cyrtoneura* Hua: identification of genes involved in polysaccharide biosynthesis. *Plant methods* 15:65. <https://doi.org/10.1186/s13007-019-0441-9>

32. Xiong F, Zhong R, Han Z, Jiang J, He L, Zhuang W, Tang R (2011) Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) genotypes. *Molecular biology reports* 38:3487-3494. <https://doi:10.1007/s11033-010-0459-6>
33. Xu YX, Shen SY, Chen W, Chen L (2019) Analysis of Genetic Diversity and Development of a SCAR Marker for Green Tea (*Camellia sinensis*) Cultivars in Zhejiang Province: The Most Famous Green Tea-Producing Area in China. *Biochem Genet* 57:555-570. <https://doi:10.1007/s10528-019-09909-1>
34. Yang S, Xue S, Kang W, Qian Z, Yi Z (2019) Genetic diversity and population structure of *Miscanthus lutarioriparius*, an endemic plant of China. *PloS one* 14:e0211471
35. Yeh F, Yang R-C, Boyle TBJ, Ye ZH, Mao JX (1997) POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta. Canada
36. Yu C, Guo H, Zhang Y, Song Y, Pi E, Yu C, Zhang L, Dong M, Zheng B, Wang H, Shen C (2017) Identification of potential genes that contributed to the variation in the taxoid contents between two *Taxus* species (*Taxus media* and *Taxus mairei*). *Tree physiology* 37:1659-1671
37. Zargar SM, Farhat S, Mahajan R, Bhakhri A, Sharma A (2016) Unraveling the efficiency of RAPD and SSR markers in diversity analysis and population structure estimation in common bean. *Saudi journal of biological sciences* 23:139-149
38. Zhang HY, Hu WC, Ma GX, Zhu NL, Sun XB, Wu HF, Xu XD (2018) A new steroidal saponin from *Polygonatum sibiricum*. *Journal of Asian natural products research* 20:586-592. <https://doi:10.1080/10286020.2017.1351436>
39. Zhang J, Xie W, Wang Y, Zhao X (2015) Potential of Start Codon Targeted (SCoT) markers to estimate genetic diversity and relationships among Chinese *Elymus sibiricus* accessions. *Molecules* 20:5987-6001. <https://doi:10.3390/molecules20045987>
40. Zhao X, Zou G, Zhao J, Hu L, Lan Y, He J (2020) Genetic relationships and diversity among populations of *Paris polyphylla* assessed using SCoT and SRAP markers. *Physiology and molecular biology of plants : an international journal of functional plant biology* 26:1281-1293. <https://doi:10.1007/s12298-020-00808-z>

Figures

Figure 1

Dendrogram of 28 accessions of *Polygonatum* amplified with SCoT markers

Figure 2

Two-dimensional plots of PCoA indicating relationships among 28 *Polygonatum* accessions based on SCoT markers

Figure 3

The results of cophenetic correlation analysis among 28 *Polygonatum* accessions based on SCoT markers

Figure 4

Values of ΔK , $K = 2$ indicates the maximum ΔK value. b: Different colors represent different populations, red represents Group I, green represents Group II

Figure 5

Provincial distribution of *Polygonatum* germplasm involved in this study