GENETIC DIVERSITY AND DNA FINGERPRINTING OF *DENDROBIUM OFFICINALE* BASED ON ISSR AND SCOT MARKERS

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Abstract. The research and utilization of germplasm resources serves as an important basis for breeding good varieties of the medicinal orchid *Dendrobium officinale*. In this study, ISSR and SCoT molecular markers were used to study the genetic diversity and genetic relationships of 24 *D. officinale* germplasms. Ten ISSR primers and 12 SCoT primers amplified 81 and 96 gene loci, of which 81 and 95 loci were polymorphic, respectively. ISSR markers gave a genetic diversity index (*H*) of 0.3388, Shannon information index (*I*) of 0.5097, and genetic distance ranging from 0.1749 to 0.9605, with an average of 0.4414; SCoT markers gave an *H* of 0.3330, *I* of 0.5043, and genetic distance ranging from 0.1823 to 0.7577, with an average of 0.4331. The results of the UPGMA cluster analysis, PCoA, genetic structure analysis, genetic distance, and geographical distance analysis of the two molecular markers were quite different. Further analysis of ISSR + SCoT showed that the combination of the two markers was complementary, and could better reveal the genetic diversity and genetic relationships of *D. officinale*. The DNA fingerprints of 24 samples of *D. officinale* were constructed based on ISSR and SCoT markers. This study provides reliable theoretical support for variety classification, breeding, and germplasm identification of *D. officinale*.
Keywords: *germplasm breeding and protection, genetic differentiation, molecular markers, polymorphism, germplasm resources*

Introduction

Dendrobium officinale (Orchidaceae) is a perennial herb that is among the rarest medicinal plants recorded in the Chinese Pharmacopoeia. It is mainly distributed in tropical and subtropical areas (Pharmacopoeia, 2020; He et al., 2022), and is cultivated in Guangxi, Guangdong, Yunnan, Zhejiang, and other regions in China. *Dendrobium officinale* contains polysaccharides, flavonoids, alkaloids, free amino acids, and other bioactive substances (Wang et al., 2021), which have diverse pharmacological effects, including immune-enhancing, anti-tumor, anti-inflammatory, antioxidant, and hypoglycemic effects (Liang et al., 2019; Kim et al., 2020; Chen et al., 2020; Tao et al., 2021), and thus has prospects for broad application in the fields of food, medicine, and health care. Wild *D. officinale* is becoming increasingly rare due to its sensitivity to changes in the living environment and its difficulty in reproduction, as well as external causes such as man-made excessive mining and environmental damage. In 1988,

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D. officinale was listed as a third-class protected species, and in 1991, it was listed as an endangered plant (Qiao et al., 2019). In recent years, with the development of the *D. officinale* planting industry, the planting area of artificial *D. officinale* has also increased. China's *D. officinale* industry chain has a certain scale, but it also has problems such as blind introduction and hybrid germplasm, resulting in large differences in the quality and yield of *D. officinale*, which affect the normal and orderly development of the industry (Chen et al., 2018).

Compared with the rapid development of the planting industry, the research on germplasm resources and variety selection of *D. officinale* started late and has developed relatively slowly. Due to the rapid development of the planting industry, wild *D. officinale* resources have been overexploited, resulting in a sharp decline in the reserves of wild *D. officinale* resources and a rapid reduction in the scope of distribution, which also has a serious impact on the systematic collection, preservation, and evaluation of *D. officinale* germplasm resources. At present, the research on the genetic basis of *D. officinale* is relatively weak, and there is a lack of basic research on the evaluation and utilization of wild resources from the perspective of breeding, resulting in a lack of theoretical support for variety breeding and improvement, and unclear breeding objectives. The collection and utilization of *D. officinale* germplasm resources is an important basis for breeding good varieties of *D. officinale* and particularly important for analysing the genetic diversity and genetic relationship of *D. officinale* germplasm resources. In this experiment, 24 wild-type and cultivated-type germplasms were collected from different regions of China for genetic diversity research and genetic relationship analysis. On this basis, DNA fingerprints were constructed for rapid detection and identification of *D. officinale* germplasms, providing a reference for the breeding research of *D. officinale*.

Due to the long-term cross-pollination and natural selection in the evolutionary process, *Dendrobium* species are highly evolved, showing a richly diverse genetic background that has resulted in morphological similarities among species, but large differences in genotypes. In the phylogenetic research into *Dendrobium*, it is difficult to judge the relationships between species by phenotypic analyses based on morphological characteristics (Song et al., 2016). Fortunately, molecular markers can effectively identify differences in plant genotypes. Inter simple sequence repeats (ISSR) is a molecular marker technology based on the principle of microsatellite repeats. This technology has the advantages of being low cost, fast, and accurate (Shen et al., 2006; Azevedo et al., 2011). Furthermore, studies have shown that ISSR molecular markers have high polymorphism in *D. officinale* (Shen et al., 2006; Ding et al., 2009), which can be effectively applied to the study of its genetic diversity. On the other hand, start codon targeted (SCoT) markers analysis is based on the principle that there is a short conservative sequence on the side of the translation start site ATG in genomic DNA for single primer amplification (Collard and Mackill, 2008). SCoT has the same advantages as ISSR, as well as amplifying the bands associated with functional genes and reflecting the polymorphism of related functional genes. SCoT has been applied to the genetic diversity (Tabasi et al., 2020; Gogoi et al., 2020), genetic relationship (Zhao et al., 2020; Zarei and Erfani-Moghadam, 2021), and DNA fingerprint construction of many plants (Saboori et al., 2019; Gupta et al., 2021).

There are usually some differences in the results of genetic diversity obtained by using a single molecular marker technology (Feng et al., 2015; Gupta et al., 2021; Khodaee et al., 2021). Therefore, this study used 24 *D. officinale* germplasms as experimental materials on which to carry out genetic structure analysis at the molecular level through

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ISSR and SCoT molecular marker technology. The study then comprehensively compared the analysis results of the two molecular markers and analyzed the feasibility of their application to *D. officinale*. Based on this study, the DNA fingerprints of *D. officinale* were constructed, which provides theoretical guidance for the protection and utilization of *D. officinale* germplasm resources, variety identification and classification, breeding, and improvement (Feng et al., 2015; Gupta et al., 2021; Khodaee et al., 2021).

Materials and methods

Experimental materials

The relevant information on the 24 *D. officinale* germplasms is shown in *Table 1*. SCoT primers were SCoT molecular marker primers scot-1-scot-36, published by Collard and Mackill (2008). ISSR primers were ISSR molecular marker primers issr-801-issr-860, published by the University of British Columbia.

Experimental method

Genomic DNA extraction

The fresh tender leaves of the plants were collected uniformly, and the genomic DNA was extracted by the improved CTAB method. The quality was analyzed by 1% agarose gel electrophoresis, and the DNA extraction solution was diluted to 40 ng μL^{-1} for the amplification reaction. The remaining DNA was stored at -20℃ for standby.

PCR amplification and detection

The PCR amplification procedures for ISSR and SCoT markers were set as follows: pre-denaturation at 94℃ for 3 min, denaturation at 94℃ for 30 s, renaturation at 57℃ for 30 s, extension at 72℃ for 30 s, 35 cycles, total extension at 72℃ for 5 min. The reaction system (20 UL) included 1 μL DNA template (40 ng μL^{-1}), 1.2 μL primers (10 μMol μL^{-1}), 10 μL mix mixture and 7.8 μL ddH_2O. 5 μL of the amplification products and 1 μL 6 × Glycerol DNA loading buffer were mixed and detected by agarose gel electrophoresis (120V, 40-60 min). The gel imaging system was used to take photos and save the results.

Primer screening

Primers with good amplification effects, clear backgrounds, and high polymorphism were selected from 60 ISSR primers and 36 SCoT primers for the molecular markers.

Data processing

According to the PCR amplification results of the ISSR and SCoT markers, the number and location of bands were converted into 0 and 1 data, and the presence or absence of DNA amplification bands at the same site under the same mobility was recorded as “1” and “0”, respectively. The genetic diversity index (h, I), genetic similarity coefficient, and genetic distance of 24 *Dendrobium candidum* germplasms were calculated by POPGENE 32 software, the genetic structure was analyzed by STRUCTURE (developed by Pritchard Laboratory of Stanford University), and the cluster analysis map was drawn in MEGA7. Geographical distance was calculated in RStudio (Wei et al., 2022) (R Language Pack used : geosphere, ape, ade4, ecodist, ggplot2, ggpubr, ggpmisc, dplyr) and the Mantel test was performed.

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Table 1. *germplasm information and phenotype statistics*

Germplasm number	Germplasm nomenclature	Source	Germplasm category	Stem thickness	Stem length	Internode	Stem color	Leaf color
1	C4	Guangdong Zhanjiang Academy of Agricultural Sciences	Cultivated Germplasm	crude	moderate	short	green	dark green
2	Zjm	Guangdong Zhanjiang Academy of Agricultural Sciences	Cultivated Germplasm	moderate	long	moderate	green	green
3	Zhj	Guangdong Zhanjiang Academy of Agricultural Sciences	Cultivated Germplasm	fine	short	moderate	purple	green
4	YSY	Guangdong yongshengyuan company	Cultivated Germplasm	fine	long	moderate	green	green
5	T1194	Shaoguan, Guangdong	Wild Germplasm	crude	moderate	short	purple	purple
6	ZK2	Gaozhou City, Guangdong Province	Wild Germplasm	crude	moderate	long	green	green
7	Gz	Guangzhou, Guangdong	Wild Germplasm	fine	long	moderate	green	green
8	GZL	Guangzhou, Guangdong	Wild Germplasm	fine	short	moderate	green	green
9	W	Guangxi Yulin Yangping Dendrobium Institute	Cultivated Germplasm	moderate	long	moderate	purple	purple
10	XL1	Xilin County, Guangxi	Cultivated Germplasm	crude	moderate	moderate	green	green
11	XL2	Xilin County, Guangxi	Cultivated Germplasm	crude	moderate	moderate	purple	purple
12	SL	Xilin County, Guangxi	Wild Germplasm	crude	moderate	moderate	purple	green
13	T1191	Guiping, Guangxi	Wild Germplasm	fine	long	moderate	green	green
14	GPT	Guiping, Guangxi	Wild Germplasm	moderate	moderate	moderate	purple	green
15	H	Guiping, Guangxi	Wild Germplasm	fine	long	moderate	brown	brown
16	L-8	Guiping, Guangxi	Wild Germplasm	thin at the bottom and thick at the top	moderate	moderate	brown	green
17	Gjh001	Rong County, Guangxi	Wild Germplasm	moderate	moderate	moderate	yellowish green	dark green
18	T119	Rong County, Guangxi	Wild Germplasm	thick at the bottom and thin at the top	moderate	moderate	purple	purple
19	ZY	Guilin, Guangxi	Wild Germplasm	crude	moderate	short	purple	purple

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Germplasm number	Germplasm nomenclature	Source	Germplasm category	Stem thickness	Stem length	Internode	Stem color	Leaf color
20	LP	Lipu County, Guangxi	Wild Germplasm	crude	moderate	long	green	green
21	RT3	Yulin, Guangxi	Wild Germplasm	crude	moderate	long	green	green
22	BB	Bobai County, Guangxi	Wild Germplasm	fine	moderate	moderate	purple	green
23	DH	Yunnan Dehong Tropical Agricultural Science Research Institute	Cultivated Germplasm	fine	short	moderate	green	green
24	GN	Yueqing City, Zhejiang Province	Wild Germplasm	fine	moderate	short	purple	purple

Note: The classification standards of stem diameter and stem length are as follows: stem diameter: thick (more than 6 mm), moderate (4-6 mm), thin (less than 4 mm). Stem length: long (more than 20 cm), moderate (10-20 cm), short (less than 10 cm)

Results and analysis

ISSR and SCoT amplified polymorphism analysis

From 60 ISSR primers and 36 SCoT primers, 10 and 12 primers with clear background, good stability, and rich polymorphism were screened, respectively (*Tables 2 and 3*). The amplification results of some of the primers are shown in *Figure 1 and Figure 2*. A total of 81 gene loci were amplified by 10 ISSR primers in the 24 *D. candidum* germplasms, including 81 polymorphic loci, accounting for 100% of the total loci. The average number of loci amplified by each primer was 8.1, the average number of alleles (N_a) was 2.0000, the average number of effective alleles (N_e) was 1.5797, Nei's gene diversity index (H) was 0.3388, and the Shannon information index (I) was 0.5790. A total of 96 gene loci were amplified from 24 *D. officinale* germplasms by 10 SCoT primers, including 95 polymorphic loci, accounting for 98.7% of the total loci. An average of 8.0 loci were amplified by each primer. The average N_a was 1.9896, the average N_e was 1.5545, H was 0.3330, and I was 0.5043. The genetic diversity index results of the two molecular markers indicated that all 24 *D. officinale* germplasms had rich genetic diversity.

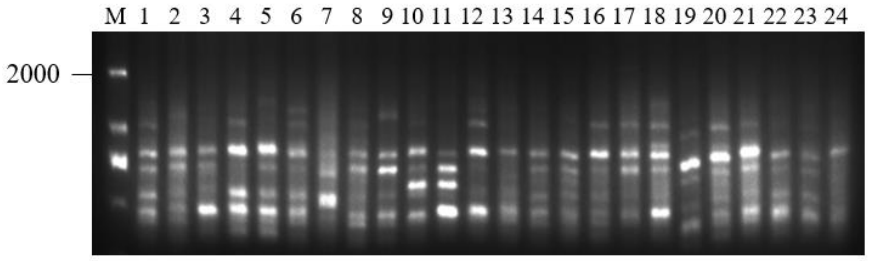

Figure 1. *ISSR-PCR amplification of 24 Dendrobium officinale varieties with primer issr-840, M: DL2000 DNA marker; 1-24: germplasm numbers of 24 D. officinale*

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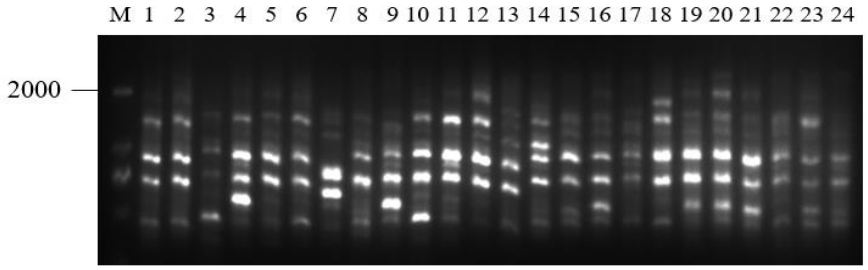

Figure 2. *SCoT PCR amplification of 24 Dendrobium officinale varieties with primer scot-31, M: DL2000 DNA marker; 1-24: germplasm numbers of 24 Dendrobium officinale*

Table 2. *Polymorphism analysis of ISSR marker primers*

Primer name	Annealing temperature (℃)	Total gene loci	Polymorphic loci	Polymorphism ratio (%)	Number of observed alleles (N_a)	Number of effective alleles (N_e)	Gene diversity index (H)	Shannon information index (I)
Issr-807	57	9	9	100	2.0000	1.7999	0.4371	0.6268
Issr-810	51	7	7	100	2.0000	1.5606	0.3413	0.5182
Issr-824	51	7	7	100	2.0000	1.7484	0.4117	0.5967
Issr-826	51	11	11	100	2.0000	1.6212	0.3592	0.5333
Issr-834	55	6	6	100	2.0000	1.5127	0.3038	0.4652
Issr-840	47	8	8	100	2.0000	1.4471	0.2756	0.4332
Issr-841	57	9	9	100	2.0000	1.5672	0.3287	0.4956
Issr-844	59	7	7	100	2.0000	1.3728	0.2614	0.4252
Issr-850	49	7	7	100	2.0000	1.4215	0.2560	0.4042
Issr-855	61	10	10	100	2.0000	1.6445	0.3681	0.5449
total		81	81					
average value		8.1	8.1	100	2.0000	1.5797	0.3388	0.5097

Table 3. *Polymorphism analysis of SCoT labeled primers*

Primer name	Annealing temperature (℃)	Total gene loci	Polymorphic loci	Polymorphism ratio (%)	Number of observed alleles (N_a)	Number of effective alleles (N_e)	Gene diversity index (H)	Shannon information index (I)
SCoT-2	50	9	9	100	2.0000	1.7027	0.4051	0.5923
SCoT-3	48	7	7	100	2.0000	1.2995	0.2193	0.3696
SCoT-14	53	8	7	87.5	1.8750	1.2769	0.1966	0.3261
SCoT-15	54	7	7	100	2.0000	1.8613	0.4578	0.6491
SCoT-18	59	7	7	100	2.0000	1.7992	0.4410	0.6322
SCoT-21	57	9	9	100	2.0000	1.3984	0.2759	0.4440
SCoT-22	57	6	6	100	2.0000	1.8381	0.4554	0.6478
SCoT-27	57	8	8	100	2.0000	1.5592	0.3355	0.5088
SCoT-28	57	7	7	100	2.0000	1.3739	0.2585	0.4182
SCoT-29	57	11	11	100	2.0000	1.7514	0.4176	0.6047
SCoT-31	53	10	10	100	2.0000	1.4605	0.3039	0.4769
SCoT-35	61	7	7	100	2.0000	1.3428	0.2267	0.3736
total		96	95					
average value		8	7.9	98.7	1.9896	1.5545	0.3330	0.5043

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Genetic similarity coefficient and genetic distance analysis of D. officinale germplasm

The molecular marker data of 24 *D. officinale* germplasms were calculated and analyzed by PopGen32. The calculated results of the genetic similarity coefficient and genetic distance are shown in *Figure 3*. The genetic similarity coefficient based on ISSR molecular markers ranged from 0.3827 to 0.8395, with an average of 0.6464, and the genetic distance ranged from 0.1749 to 0.9605, with an average of 0.4414. Among them, No. 3 and No. 12 were the closest and No. 23 and No. 5 were the farthest. The genetic similarity coefficient based on SCoT molecular markers ranged from 0.4688 to 0.8333, with an average of 0.6525, and the genetic distance ranged from 0.1823 to 0.7577, with an average of 0.4331. Among them, No. 1 and No. 2 were the closest, and No. 23 and No. 24 were the farthest. The genetic similarity coefficient based on ISSR + SCoT molecular markers ranged from 0.4802 to 0.7910, with an average of 0.6497, and the genetic distance ranged from 0.2345 to 0.7335, with an average of 0.4348. Among them, No. 3 and No. 12 were the closest, and No. 7 and No. 23 were the farthest.

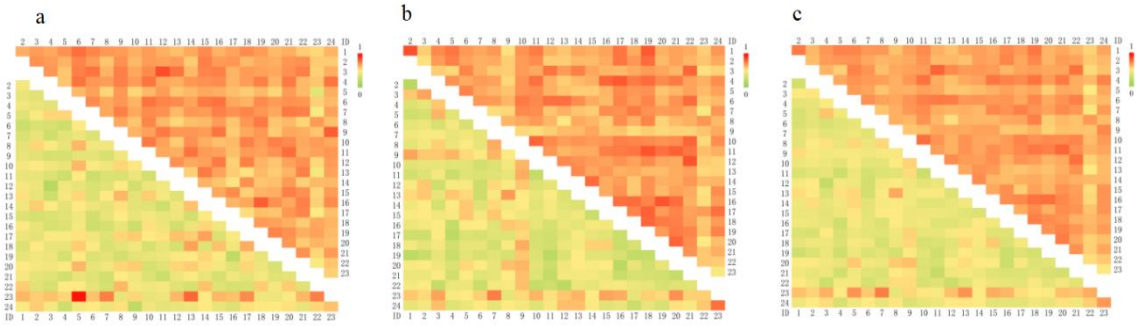

Figure 3. *Genetic similarity coefficient and genetic distance matrix heat maps for (a) ISSR, (b) SCoT, and (c) ISSR + SCoT. Note: The genetic similarity coefficient is above the diagonal, and genetic distance is below the diagonal; 1-24 are germplasm numbers of 24 Dendrobium officinale individuals*

Cluster analysis based on ISSR and SCoT markers

Using the molecular marker data for the 24 *D. officinale* germplasms, UPGMA cluster analysis and mapping were carried out. Based on the cluster analysis results for the ISSR markers, the 24 *D. officinale* germplasms could be divided into four groups (*Fig. 4a*), in which germplasms No. 5, 8, 17, and 23 constituted a group far away from the other 20 germplasms, which were divided into two subgroups within the first group. According to the results of the cluster analysis based on SCoT markers, the 24 *D. officinale* germplasms could be divided into three groups (*Fig. 4b*). Among them, germplasms 8, 9, and 23 constituted a group far away from the other 21 germplasms, and these 21 germplasms were also divided into two subgroups within the first group. The results of the UPGMA cluster analysis of the germplasms showed that there were significant differences between the two molecular markers. By further combining the results of ISSR and SCoT molecular markers for UPGMA cluster analysis (*Fig. 4c*), the germplasms were divided into 3 groups, and the clustering results integrated the clustering results of the two molecular markers, which had more reference significance in revealing the genetic background of *D. officinale*.

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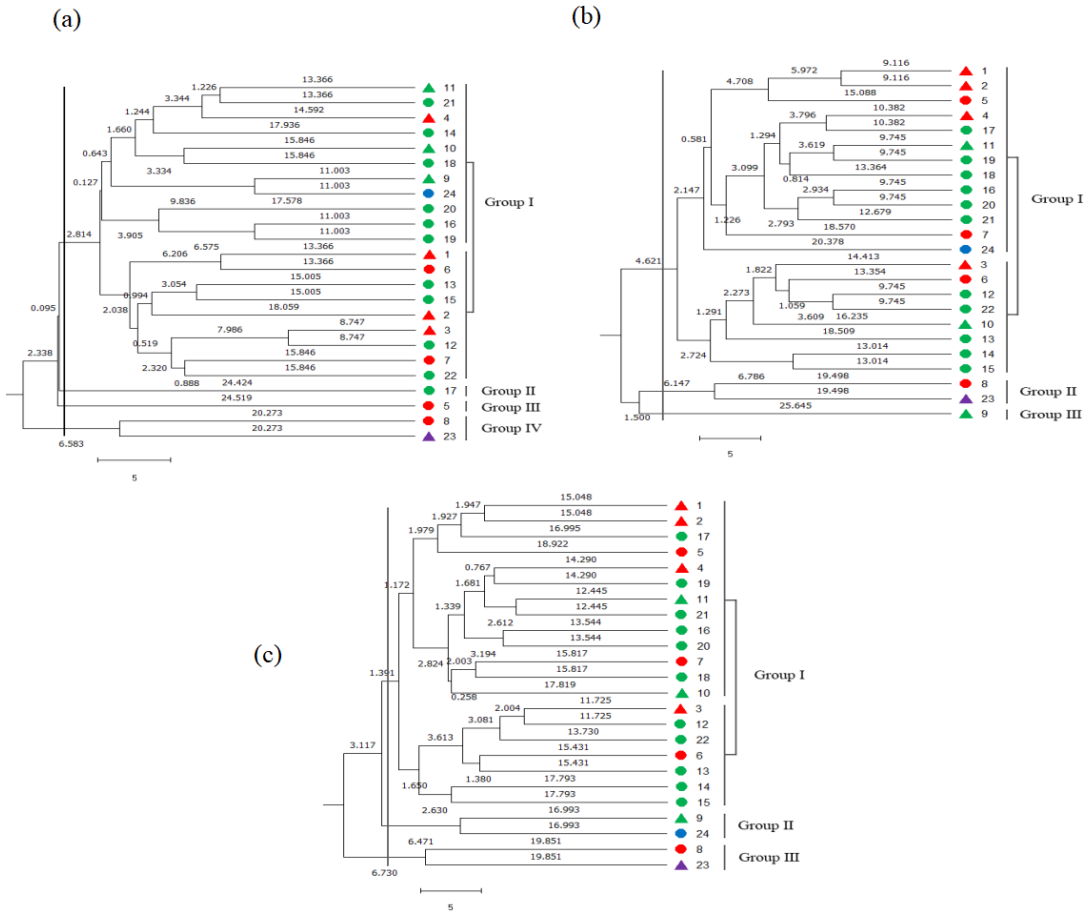

Figure 4. *UPGMA cluster analysis diagram of (a) ISSR, (b) SCoT, and (c) ISSR + SCoT. Note: 1-24 are the numbers of the 24 Dendrobium officinale germplasms. Red is Guangdong, green is Guangxi, blue is Zhejiang, and purple is Yunnan. Circles denote wild-type germplasm and triangles denote cultivated germplasm*

Principal coordinate analysis and Mantel test based on ISSR and SCoT

Principal coordinate analysis (PCoA) was conducted based on the genetic distance of the 24 *D. officinale* germplasms. According to the data analysis of the ISSR markers, the variation of the horizontal and vertical coordinate axes was 12.98% and 8.86%, respectively (*Fig. 5a*). The germplasms were staggered in the two-dimensional coordinate map. The distribution of germplasms in Guangxi was relatively scattered, and the germplasms in Guangdong were basically within the distribution area of the germplasms in Guangxi. The results did not show a clear distribution law. According to the data analysis of the SCoT marker, the variation of the horizontal and vertical coordinate axes was 13.45% and 11.55%, respectively (*Fig. 5b*). The classification of various qualities in the coordinate graph was clearer and more intuitive than the results of ISSR, which could obviously cluster the germplasms with similar genetic backgrounds. According to the data analysis of ISSR + SCoT markers, the variation of the horizontal and vertical coordinate axes was 10.69% and 9.03%, respectively (*Fig. 5c*). This result is similar to the distribution of SCoT germplasms. In terms of classification, some germplasms were more closely distributed than SCoT. The correlation between ISSR and SCoT marker results was analyzed by Mantel test. The results showed a significant correlation between ISSR

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and SCoT marker results (r = 0.2632 and p = 0.011 (*Fig. 6a*)), but the correlation coefficient was low. However, Mantel analysis showed that the correlation coefficients of ISSR + SCoT and ISSR (r = 0.7304, p = 0.001 (*Fig. 6b*)) and ISSR + SCoT and SCoT (r = 0.8505, p = 0.001 (*Fig. 6c*)) were both highly significant.

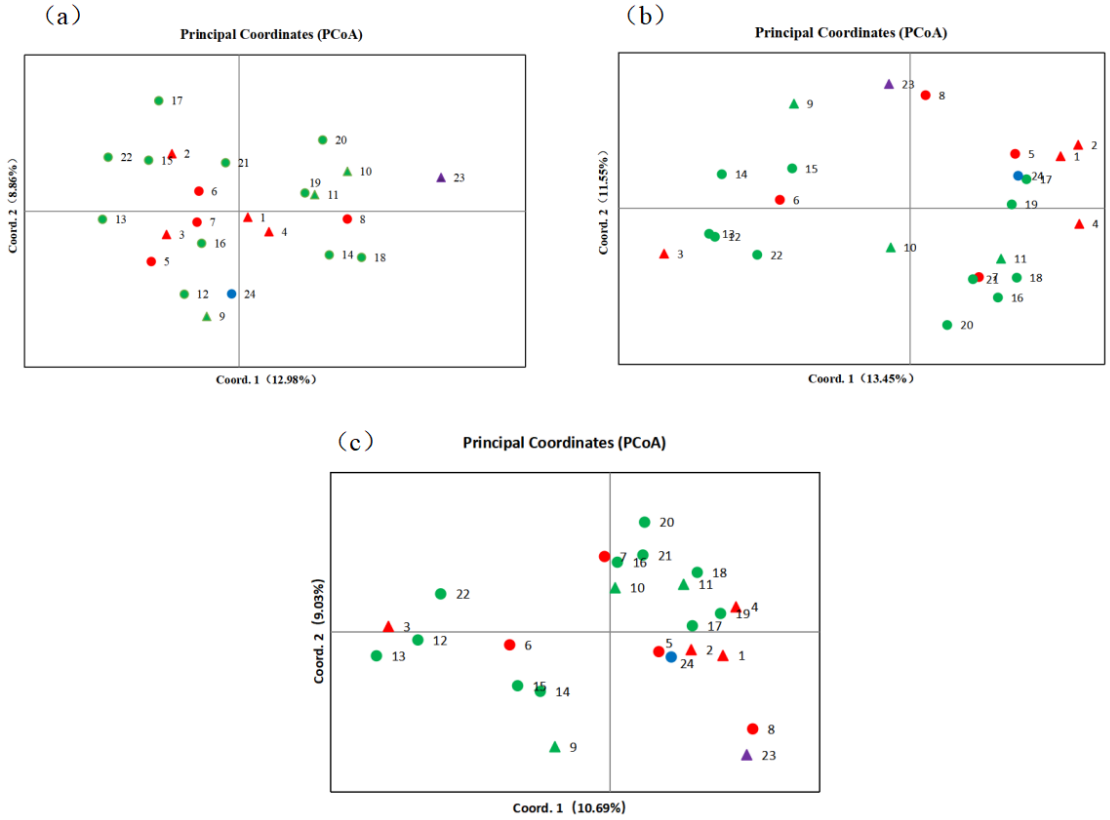

Figure 5. *Coordinate diagram of PCoA based on genetic distance for (a) ISSR, (b) SCoT, and (c) ISSR + SCoT. Note: 1-24 are the numbers of the 24 D. officinale germplasms. Red is Guangdong, green is Guangxi, blue is Zhejiang, and purple is Yunnan. Circles denote wild-type germplasm and triangles denote cultivated germplasm*

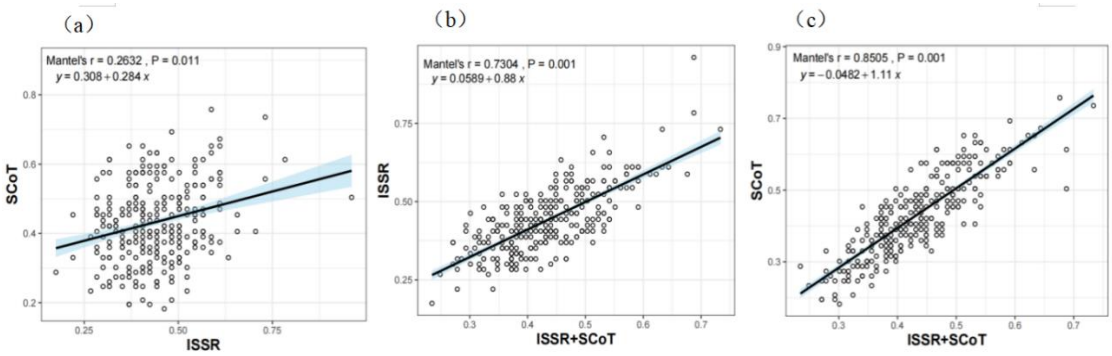

Figure 6. *Mantel analysis of ISSR, SCoT, and between ISSR and SCoT, (a) ISSR and SCoT (b) ISSR + SCoT and ISSR (c) ISSR + SCoT and SCoT*

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Genetic structure analysis

The genetic structure of the 24 samples of *D. officinale* was analyzed by structure software. The range of grouping number *K* value was set to 1-10. Each *K* value was simulated for 5 times, and the relationship between *K* value and delta *K* was drawn. The structure software was based on a Bayesian model to analyze the population structure. When the delta *K* value reached the maximum, the *K* value was the best population number. It can be seen from *Figure 7* that when the maximum delta *K* value is 1.93 according to ISSR, *K* = 2, when the maximum delta *K* value is 15.83 according to scot, *K* = 2, and when the maximum delta *K* value is 157 according to ISSR + SCoT, *K* = 3. *Figure 8* shows the genetic structure analysis of the *D. officinale* germplasms. The results of the ISSR model operation showed that the 24 germplasms were not clearly divided into groups, while the results of the SCoT, and ISSR + SCoT, model operations divided the 24 germplasms into 2 and 3 groups, respectively, and showed some gene exchange between the germplasms. The running results of the three kinds of data were consistent with the classification results of the PCoA, which further indicates that ISSR + SCoT is more refined in germplasm classification and could better reveal the genetic backgrounds of the *D. officinale* germplasms.

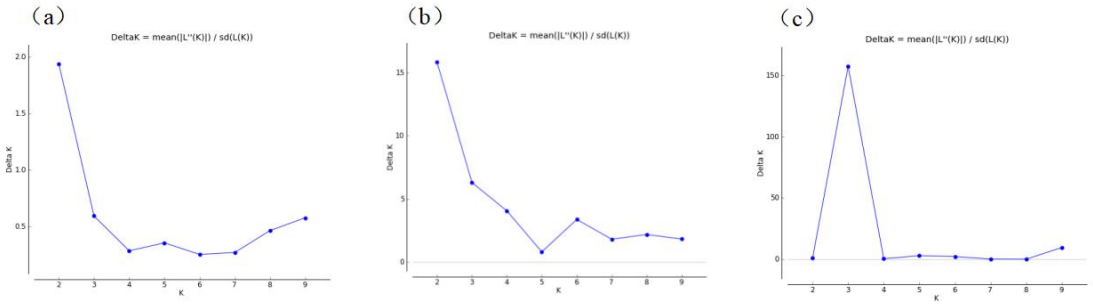

Figure 7. *K-value curve of genetic structure analysis for (a) ISSR, (b) SCoT, and (c) ISSR + SCoT*

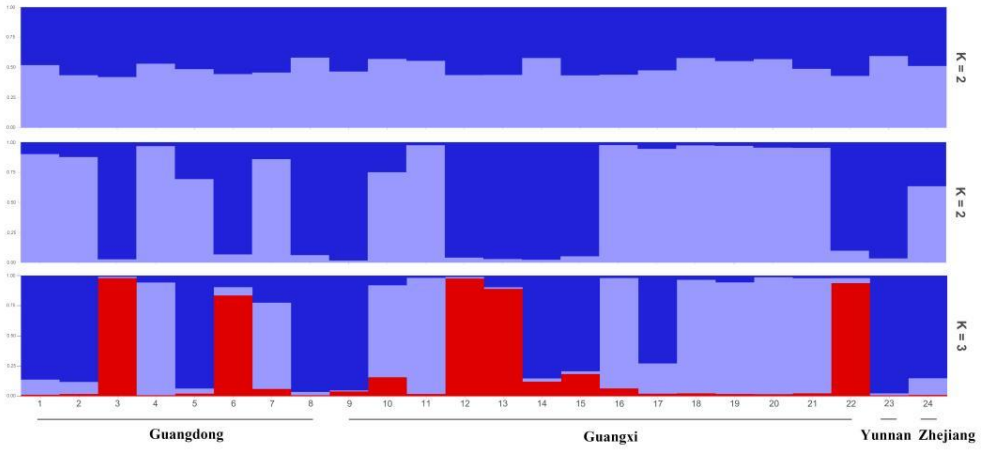

Figure 8. *Genetic structure analysis of 24 Dendrobium officinale germplasms using (a) ISSR, (b) SCoT, and (c) ISSR + SCoT. Note: 1-24 are the numbers of the 24 D. officinale germplasms*

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Mantel analysis of genetic and geographical distance

Using the longitude and latitude information for the 24 *D. officinale* germplasms, the geographical distance between the different germplasms was calculated. A Mantel analysis of genetic distance and geographical distance was carried out using RStudio. The results for the ISSR markers showed that r = 0.2648 and p = 0.033 (*Fig. 9a*), and the results for the SCoT markers showed that r = 0.2185, p = 0.073 (*Fig. 9b*). The correlation coefficients between genetic distance and geographical distance based on ISSR and SCoT markers were low; however, the genetic distance of ISSR markers was significantly correlated with geographical distance. The correlation analysis between genetic distance and geographical distance based on ISSR + SCoT markers showed that r = 0.3002 and p = 0.022 (*Fig. 9c*), showing a significant correlation, and the R value was higher than that of ISSR, with a higher correlation coefficient.

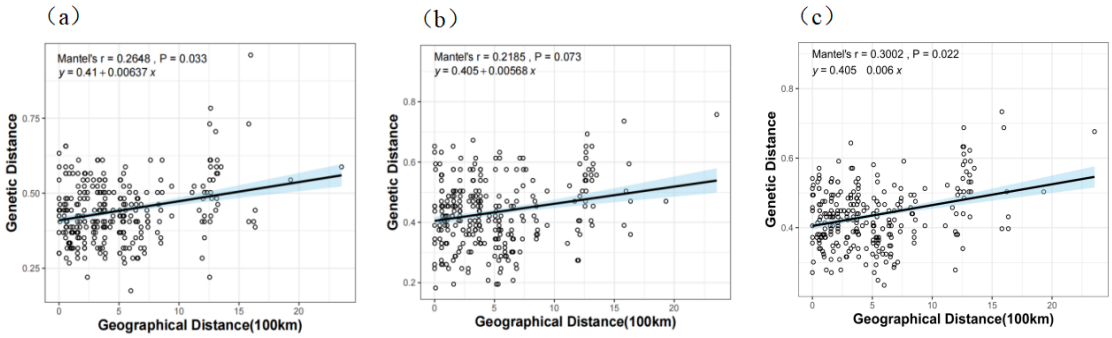

Figure 9. *Mantel analysis of genetic distance and geographical distance for (a) ISSR, (b) SCoT, and (c) ISSR + SCoT*

Construction of DNA fingerprints of 24 Dendrobium officinale germplasms

In order to distinguish between the 24 samples of *D. officinale*, the minimum marker combinations were screened from the ISSR and SCoT molecular marker data. The results showed that two ISSR primer combinations (issr-826, 855) (*Fig. 10a*) and two SCoT primer combinations (scot-3, 4) (*Fig. 10b*) could be used to construct DNA fingerprints of the 24 samples of *D. officinale*.

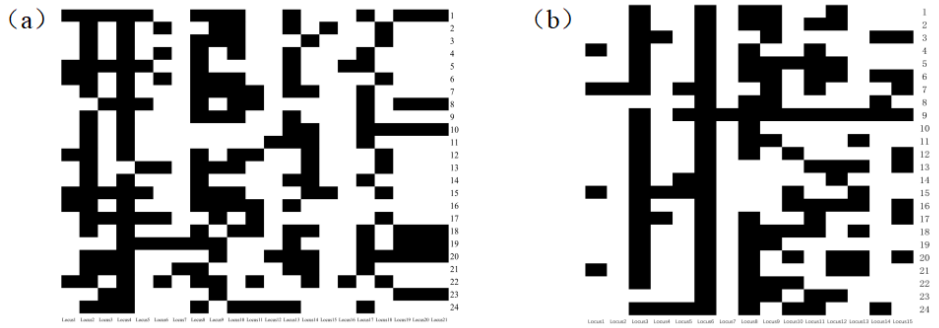

Figure 10. *DNA fingerprints of 24 Dendrobium candidum germplasms using (a) ISSR, and (b) SCoT. Note: 1-24 are the numbers of the 24 D. officinale germplasms*

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Discussion

Comparison of ISSR and SCoT

Research into the genetic diversity of germplasm is important to understanding the genetic variation and genetic relationship of germplasm resources. It is often impossible to accurately understand the genetic relationships of *D. officinale* and distinguish between different germplasms through phenotypic information (Lu et al., 2019). ISSR and SCoT molecular markers are amplified from DNA to obtain gene locus information, which can better distinguish the genotype of germplasm, and play an important role in revealing the genetic background of germplasm resources (Yilmaz and Ciftci, 2021). The results of this study showed that ISSR and SCoT molecular markers could amplify more abundant polymorphic loci, with an average percentage of polymorphic loci of 100% and 98.7%, and Nei's gene diversity index of 0.3388 and 0.3330, respectively. Both ISSR and SCoT markers could be effectively applied to the study of genetic diversity of *D. officinale*. Because the primer sequence of SCoT molecular markers is distributed near the translation start site on the genome, it can amplify more sites associated with functional genes, while the primer of ISSR is a simple repetitive sequence, with greater randomness, and is a dominant marker. Furthermore, marking efficiency will differ in different species (Ibrahim, 2021; Zhao et al., 2021). In this study, UPGMA cluster analysis, PCoA, genetic structure analysis, the Mantel test, and other methods were used to comprehensively compare the results of the two methods. The results showed that there were significant differences between ISSR and SCoT markers in the 24 *D. officinale* germplasms, indicating that the genetic locus information obtained by the two molecular markers were different. Therefore, comprehensive analysis of multiple molecular markers should be used as much as possible in the evaluation of the genetic diversity of *D. officinale* in order to obtain more accurate and reliable research results. The number of polymorphic loci amplified by the SCoT markers was significantly more than that of the ISSR markers. Therefore, in cluster analysis and PCoA, the SCoT markers could more clearly and intuitively describe the genetic background and genetic relationships between *D. candidum* germplasm, which also makes the SCoT marker more advantageous in the study of genetic diversity of the plant, which is consistent with the results of ISSR and SCoT application studies of *Camellia oleifera* (Xiao, 2019) and *Celastrus orbiculatus* (Yang et al., 2022). Nevertheless, ISSR markers also play an important role in revealing the genetic structure and genetic relationships of plants (Ding et al., 2016; Cao and Hauk, 2022). In this study, the ISSR results showed that there was a significant correlation between genetic distance and geographical distance among germplasms, and there was no significant correlation between genetic distance and geographical distance in the correlation analysis of SCoT, while the analysis results based on ISSR + SCoT were consistent with the results of ISSR, showing a significant correlation. It can be seen that the combination of the two markers can complement each other and better reveal the genetic diversity of samples. Mantel analysis of SCoT, ISSR, and ISSR + SCoT showed that the correlation coefficient between SCoT and ISSR markers was low, while the correlation coefficient between ISSR + SCoT and SCoT, and ISSR + SCoT and ISSR, was high, showing a very significant correlation. These results further verified that ISSR and SCoT were complementary, which was also consistent with the results of Safari et al. (2019) and Dias et al. (2019).

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Genetic diversity assessment of Dendrobium officinale

The 24 samples of *D. officinale* in this study were from Guangxi, Guangdong, Yunnan, and Zhejiang provinces in China, including 8 cultivated germplasms and 16 wild germplasms. Based on the results of ISSR and SCoT analysis, we used ISSR + SCoT to evaluate the genetic diversity of *D. officinale*. The genetic similarity coefficient of the 24 *D. officinale* samples ranged from 0.4802 to 0.7910, with an average of 0.6497; the genetic distance ranged from 0.2345 to 0.7335, with an average of 0.4348; *H* was 0.3359, and *I* was 0.5070, indicating that there were significant genetic differences among the samples. This indicated that the germplasms of the species had rich genetic diversity and could be used as a reserve of breeding materials. According to the genetic structure analysis, the best group of 24 germplasms was divided into three groups, and the divisions were basically consistent with the results of the PCoA. However, in the UPGMA cluster analysis, there were some differences between the division results of a small number of germplasms and the results of the genetic structure analysis, which may have been caused by the different algorithms used in the two analysis methods (Xu et al., 2007). The software used for genetic structure analysis can reduce the influence of human factors on the classification of groups, and then correct the genetic structure of the material population, making the classification of groups more meticulous (Xu et al., 2020). Therefore, the classification of the population structure of germplasm resources obtained from the analysis of a structure model can be used as an effective supplement to the results of UPGMA clustering analysis, which can be used for reference. Although the 24 *D. officinale* samples were not grouped strictly according to their geographical origin or phenotypic traits, the results clearly showed the genetic relationships between these germplasms, which provide the necessary theoretical support for breeding research (Xu et al., 2007, 2020).

Utilization of Dendrobium candidum germplasm resources

Germplasm resources are not only important materials in biological research, but also the raw materials for breeding new crops and varieties. Genetic diversity analysis of breeding materials requires high marker polymorphism, large amounts of locus information, and the ability to cover the entire genome evenly, so as to accurately reflect the genetic relationship between germplasm materials and provide accurate reference information for breeding plans. Evidently, ISSR and SCoT markers can meet this requirement (Costa et al., 2020; Buer et al., 2022). Because the SCoT marker itself may be a part of the target gene or closely linked with the target gene, and can be distributed near the transcription region, the utility of screening available traits in breeding will be higher than that of ISSR marker, which can accelerate the breeding process (Luo et al., 2011; Wu et al., 2013; Etminan et al., 2016), while the characteristic of ISSR amplification in non-coding regions makes it difficult to link with the target trait, and its application utility in breeding will be lower than that of SCoT (Igwe et al., 2021). *Dendrobium officinale* is usually bred by crossing with the phenotypic traits of more stems, stem diameter, low cellulose, high polysaccharide content, strong stress resistance, and high yield, so as to screen for germplasm with a variety of desirable traits. Through the analysis of the genetic diversity of the 24 *D. officinale* germplasms and the investigation and statistics of some phenotypes, we can intuitively understand the genetic structure of each germplasm and the genetic relationships between germplasms, and can configure the parents from the molecular level, which provides a reference for the rapid

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selection of breeding objectives. Most of the 24 *D. officinale* germplasms had distant genetic relationships, which is better for using heterosis to obtain new varieties with good parental characteristics. For example, the phenotypic character of wild germplasm No. 22, collected in the Bobai area of Guangxi, is that the number of stems is large, but the stems are relatively thin. Therefore, germplasms No. 1, No. 5, and No. 20, with distant genetic relationships and thick stems, can be selected for cross breeding, in order to obtain germplasm with good parental characteristics. The polysaccharide content of artificial cultivated germplasm No.11, from the Xilin area of Guangxi, is high. It could be hybridized with other varieties with strong growth potential, large numbers of stems, and strong stress resistance to obtain germplasm with improved characteristics.

Identification of germplasms

DNA fingerprinting code is used to identify species, and the genetic relationships between species, by using gene fragments that are representative of the species, are standard, have sufficient variation, are easy to amplify, and are relatively short. This technology has been widely used in biodiversity assessment, genetic diversity analysis, authenticity identification of plant drugs and other plant materials (Ivanovych et al., 2017; Alqahtani et al., 2017; Ghariani et al., 2019), scientific and accurate identification of the genetic specificity of crop varieties and germplasm resources, and evaluation of genetic resources. The protection of plant variety rights and interests has important practical significance. Due to the complex genetic background of *D. officinale*, the phenomenon of synonymous or homonymous foreign bodies frequently arises. It is not sufficient to rely only on morphological identification; therefore, we need to use more accurate methods to effectively distinguish between different varieties of *D. officinale*. Yang (2021) successfully constructed DNA fingerprints that can distinguish 34 *D. officinale* germplasms through SRAP, ISSR, and SSR molecular marker technologies. Cui and others (2021) used 3 ISSR primers to construct DNA fingerprints that can distinguish 22 *Dendrobium* species separately. It can thus be seen that molecular markers have become a common technical means for species identification of *Dendrobium*. In this study, ISSR and SCoT technology both amplified rich gene loci information in *D. officinale* germplasms, indicating that they both provide rapid, reliable and effective identification technology for research on *D. officinale* germplasm resources. According to the principle of using as few primers as possible to distinguish as many varieties as possible, two ISSR primer combinations (issr-826, 855) and two SCoT primer combinations (scot-3, 4) were selected from 10 ISSR primers and 12 SCoT primers suitable for *D. candidum*, and 24 DNA fingerprints of *D. candidum* were constructed, which can be used for future rapid identification of these germplasms.

Conclusion

In this study, ISSR and SCoT techniques were used to analyze the genetic characteristics and genetic relationships of 24 *D. officinale* germplasms from different regions in China. The results showed that the germplasms had rich genetic diversity. Cluster analysis, PCoA, and genetic structure analysis based on ISSR, SCoT, and ISSR + SCoT data showed that ISSR and SCoT obtained different genetic locus information in the germplasms. Both of the molecular markers could be effectively applied to the study of genetic diversity of *D. officinale*, but their classification results were quite different. The results of the ISSR markers were correlated with geographical distance, and thus

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have certain advantages for the study of germplasm distributed in different geographical locations. The clustering grouping of the germplasms by SCoT was more clear and intuitive, and its principle of association with phenotypic traits is more suitable for breeding research. The marker data based on ISSR + SCoT integrated the respective advantages of ISSR and SCoT, which made the genetic background and genetic relationships of the 24 *D. officinale* germplasms more accurate and detailed, and was more conducive to the analysis of genetic characteristics. Researchers can select the corresponding molecular markers according to their different needs in future studies of *D. officinale* germplasm resources. Furthermore, 24 DNA fingerprints of *D. officinale* were constructed using two ISSR primer combinations (issr-826, 855) and two SCoT primer combinations (scot-3, 4), respectively, which provided a scientific method for the identification of *D. officinale* germplasm. The results provide reliable theoretical support for the revealed genetic relationships of 24 *D. officinale* germplasms, as well as for breeding and popularization of desirable varieties.

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