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硬核资源遗传多样性的 AFLP 分析

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摘要：为了解云南省硬核[*Scleropyrum wallichianum* (Wight et Arn.) Arn.]的遗传多样性, 采用 AFLP 标记分析了 7 个居群 84 份种质材料的遗传变异。结果表明, 从 64 对引物组合中挑选出多态性较好的引物 8 对, 共扩增出 1 728 条带, 其中多态性条带 1 388 条, 多态性百分率为 80.14%。硬核在物种水平的多样性指数分别为 *Na*=1.416, *Ne*=1.179, *H*=0.137, *I*=0.225, 在居群水平上分别为 *H*=0.111, *I*=0.175; 在遗传相似性系数为 0.52 时, 这些种质材料可分为 3 组, 其中易武居群具有丰富的遗传变异, 大部分的遗传变异存在于居群内, 而在 0.05 置信区间内居群间遗传变异仅为 11.5%; 居群间的遗传距离和地理距离无显著相关性(r=0.0323, P=0.5820)。因此, 硬核资源可采用就地和迁地保护策略, 以增加其遗传多样性。

关键词：硬核; AFLP; 遗传结构; 遗传多样性; 引种; 保护策略
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Genetic Diversity of *Scleropyrum wallichianum* Based on AFLP Markers

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Abstract: In order to understand the genetic diversity of *Scleropyrum wallichianum* in Yunnan, the genetic variations of eighty-six germplasms from seven populations were analyzed by using AFLP markers. Eight pairs of AFLP primers with high and stable polymorphism were selected, amplified 1 728 DNA bands, of which 1 388 bands were polymorphic, accounting for 80.14%. The genetic diversity indexes at the species level were *Na*=1.416, *Ne*=1.179, *H*=0.137, *I*=0.225, and at the population level were *H*=0.111, *I*=0.175. At similarity coefficient of 0.52, all germplasms could be divided into three groups. The population YW had abundant genetic diversity, and most of genetic variation existed within populations, while genetic variation among populations was 11.5% under confidence interval of 0.05. There was no significant correlation between genetic distance and geographical distance among populations (r=0.0323, P=0.5820). Therefore, *in situ* and *ex situ* protections would be proposed for increasing genetic diversity of *S. wallichianum* germplasms.

Key words: *Scleropyrum wallichianum*; AFLP; Genetic structure; Genetic diversity; Introduction; Conservation strategy

Scleropyrum wallichianum (Wight et Arn.) Arn. is an evergreen shrub or small trees, distributed in Guangxi, Hainan and Yunnan Provinces, China[1]. It is a potential commercial resource for extracting natural acetylenic acid, because octadec-9-ynoic was discovered in Santalaceae plants[2] and 17-octadecen-9-ynoic acid was separated from *S. wallichianum*[3]. Moreover, the nutlet of *S. wallichianum* is eaten by national minorities such as Jino nationality in Xishuangbanna[4].

According to the investigation of *S. wallichianum*

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in Xishuangbanna and Pu'er of Yunnan Province, its habitats were serious destroyed especially around the villages, urging the protection of the germplasm resources. Furthermore, as a potential commercial species, it is necessary to study on germplasm resources of *S. wallichianum*. There were abundant variations in the content of octadecenoic acid among different populations[5], and the phenotypic variation of *S. wallichianum* seeds was 93.72% within population, 6.28% among populations[6]. In addition, the content of fatty acid extracted from different varieties of *S. wallichianum* seeds varied greatly[7]. However, these studies were based on the morphology and component content, no molecular research has been conducted. In order to protect more genetic resources, it is necessary analysis the genetic information in *S. wallichianum*.

Amplified fragment length polymorphism (AFLP) markers have been widely used to investigate the genetic diversity and relationships of plant germplasm resources[8−12]. AFLP markers have a number of attractive features, the application does not require knowledge of the nucleotide sequences, highly polymorphic and readily reproducible, and the PCR-based AFLP procedure is technically simple. In addition, because AFLPs are dominant molecular markers, data inter-predation is relatively simple. In this study, the genetic diversity and relationships of 86 germplasms of *S. wallichianum* from seven natural populations in Yunnan Province were analyzed by using AFLP markers, which would provide a basis for introduction and conversation of *S. wallichianum*.

1 Materials and methods

1.1 Materials

The young leaves and barks were collected from 86 individuals of seven populations throughout the distribution range[13] of *Scleropyrum wallichianum* (Fig. 1, Table 1). The materials were dried and saved by silica gel.

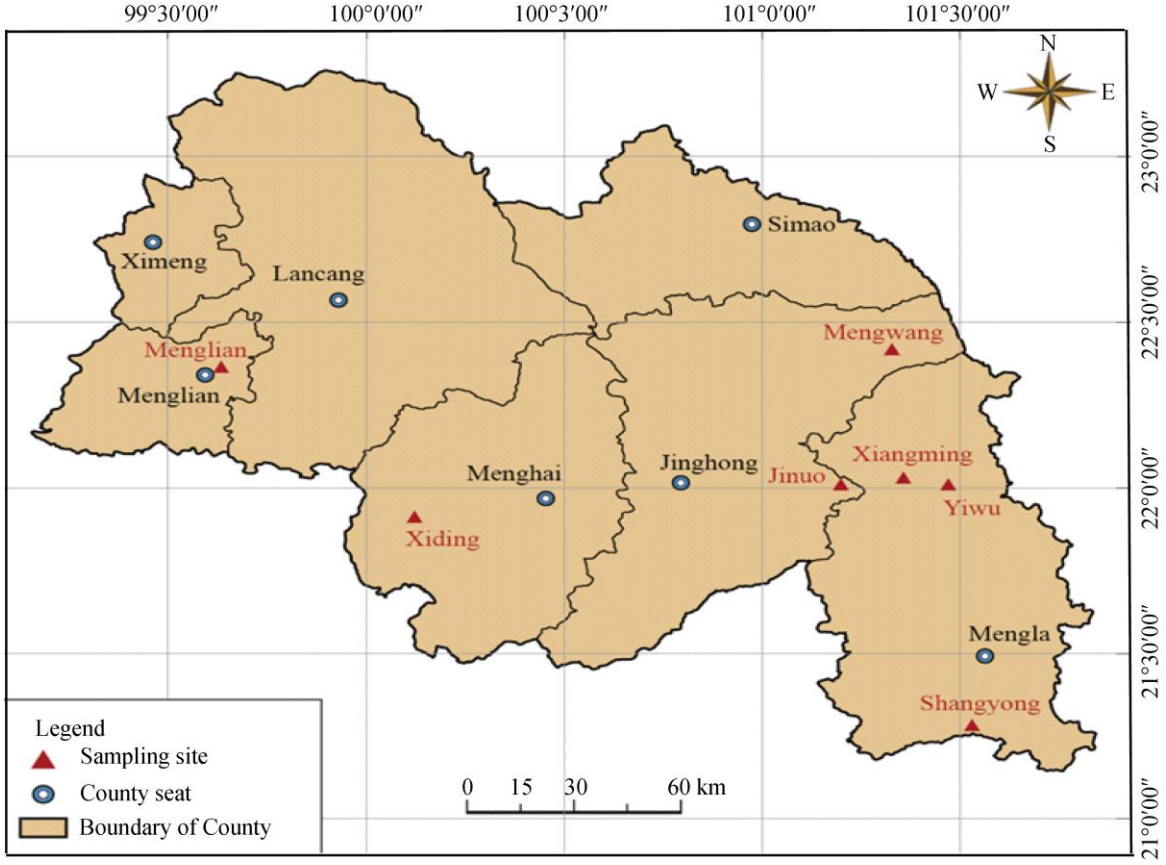

Fig. 1 Distribution of seven *Scleropyrum wallichianum* populations

Table 1 Geographic information of *Scleropyrum wallichianum*

Population	Location	Latitude (N)	Longitude (E)	Elevation (m)	Germplasm No.
JN	Jino, Jinghong, Xishuangbanna	22°00′	101°11′	1 200~1 311	JN1~JN15
YW	Yiwu, Mengla, Xishuangbanna	22°00′	101°28′	1 266~1 455	YW16~YW29
XM	Xiangming, Mengla, Xishuangbanna	22°01′	101°21′	1 079~1 439	XM30~XM44
SY	Shangyong, Mengla, Xishuangbanna	21°16′	101°31′	945~1 018	SY45~SY52
MW	Mengwang, Jinghong, Xishuangbanna	22°25′	101°19′	1 024~1 278	MW53~MW65
XD	Xiding, Menghai, Xishuangbanna	21°54′	100°07′	1 530~1 630	XD66~XD76
ML	Menglian, Menglian County	22°11′	99°38′	900~1 100	ML77~ML86

1.2 DNA extraction and AFLP analysis

Genomic DNA was extracted from dried leaves or barks by using modified cetyltrimethylammonium bromide method[14]. AFLP was performed by Beijing Dingguo Changsheng Biotechnology Co. Ltd. The genomic DNA was digested with *Eco*R I and *Mse* I restriction endonucleases.

From 64 pairs of AFLP primer, 8 pairs with high and stable polymorphism were selected, such as *E*-GAA/*M*-CAG, *E*-GAA/*M*-CTC, *E*-GAG/*M*-CAA, *E*-GAG/*M*-CAC, *E*-GAG/*M*-CAG, *E*-GAT/*M*-CAA, *E*-GAT/*M*-CAT and *E*-GAT/*M*-CTC, where *E* and *M* represent 5′-GACTGCGTACCAATTCA-3′ and 5′-GACGATGAGTCCTGAG-3′, respectively.

1.3 Data analysis

AFLP data generated using an ABI PRISM 377 sequencer were scored as "1" (presence of fragment) and "0" (absence of fragment) by GENESCAN. The binary data matrix was first analyzed using POPGENE version 1.31 under the assumption that the populations were in Hardy-Weinberg equilibrium. The parameters of genetic diversity included number of different alleles (*Na*), number of effective alleles (*Ne*), Shannon's information index (*I*), Nei's gene diversity (*H*), the total gene diversity (H_T), gene diversity within populations (H_S), coefficient of genetic variation among populations (G_{ST}), gene flow (*Nm*), Nei's genetic distance (*D*) between populations. To test the genetic differentiation among populations, analysis of molecular variance (AMOVA) was conducted using the Arlequin version 3.11[15]. The longitude and latitude were converted to geographical distance data using the longitude and latitude conversion software and the Mantel test was used to test the possible association between pairwise geographical distance and genetic distance among seven populations using TFPGA version 1.3[16].

2 Results

2.1 Genetic diversity

Out of 64 tested *Eco*R I/*Mse* I primer pairs, 8 pairs of primer showing high polymorphism were selected to evaluate and characterize the 86 germplasms of *S. wallichianum*. A total of 1 728 distinct bands were amplified, of which 1 388 bands were polymorphic, accounting for 80.14%. For each pair of primer, the number of amplified bands was 216, thus, the percentage of polymorphic bands amplified by AFLP primers ranged from 76.34% (*E*-GAA/*M*-CTC) to 84.88% (*E*-GAT/*M*-CAT). At the species level, the number of different alleles (*Na*), number of effective alleles (*Ne*), Nei's gene diversity (*H*) and Shannon's information index (*I*) were 1.417, 1.179, 0.137, and 0.225, respectively. At the population level, Nei's gene diversity (*H*) and the Shannon's information index (*I*) were estimated to be 0.111 and 0.175, respectively.

2.2 Genetic structure

When all the seven populations were considered, the coefficient of genetic variation among populations (G_{ST}) was 0.191. The total gene diversity (H_T) and gene diversity within populations (H_S) were 0.138 and 0.111, respectively. AMOVA showed that 22.7% of the total genetic variation occurred among populations and 78.3% within each population with average pairwise φ_{PT} (similar to F_{ST}) of 0.135. The estimated gene flow (*Nm*) among populations was 2.141.

2.3 Genetic relationships

At a genetic similarity coefficient of 0.52, all the germplasms were classified into three clusters in UPGMA tree, such as cluster I, II and III (Fig. 2).

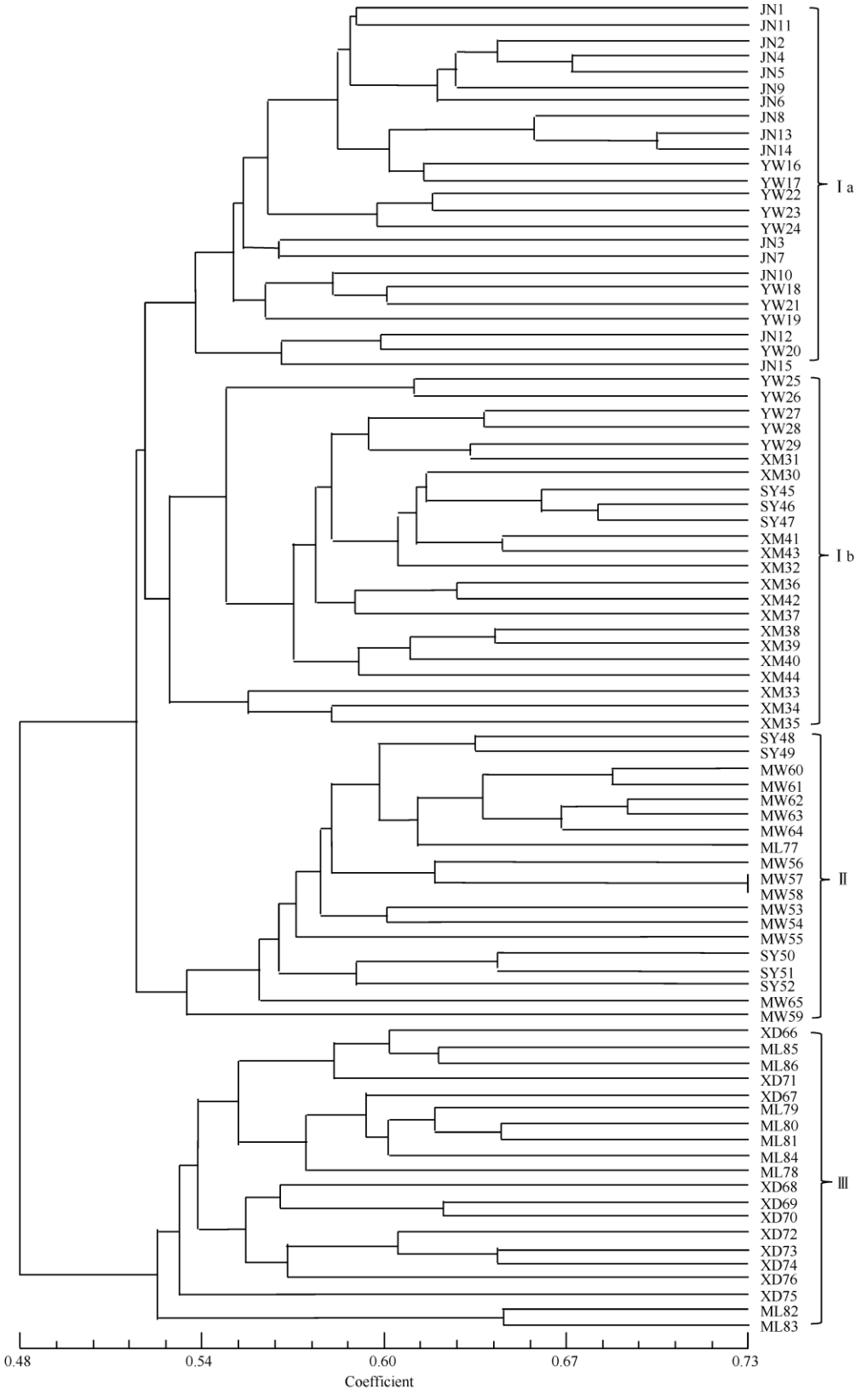

Fig. 2 UPGMA tree of *Scleropyrum wallichianum* germplasms

Cluster I was comprised of 47 germplasms, accounting for 54.65%, while cluster II and cluster III with 19 and 20 germplasms, accounting for 22.09% and 23.26%, respectively. Cluster I could be further divided into Ia and Ib groups with 24 and 23 germplasms, respectively.

Among populations, Nei's unbiased genetic identity (In) ranged from 0.9873 (between YW and XM population) to 0.936 (between JN and XD population), with an average of 0.977. The smallest genetic distance (0.015) was detected between population YW and XM, the biggest genetic distance (0.073) was detected between population SY and XM, as well as population XD and JN (0.072) (Table 2). The relationships among seven populations were analyzed based on Nei's unbiased genetic identity (In) by using UPGMA clustering method (Fig. 3). Population YW and XM were grouped into a cluster at first, following population SY and MW, then with population ML and XD again, and finally with population JN. There was no significant correlation between genetic distance and geographical distance among all populations (r=0.0323, P=0.5820) by Mantel test.

Table 2 Nei's unbiased genetic identity and genetic distance among populations

Population	JN	YW	XM	SY	MW	ML	XD
JN		0.046 3 (28.1)	0.048 8 (16.4)	0.052 1 (87.3)	0.053 1 (47.4)	0.066 2 (161.5)	0.072 3 (111.7)
YW	0.960 6		0.014 8 (12.1)	0.025 2 (80.6)	0.020 2 (48.1)	0.029 8 (189.4)	0.034 9 (139.7)
XM	0.957 8	0.987 3		0.072 8 (84.5)	0.022 3 (43.5)	0.027 0 (177.3)	0.031 8 (128.1)
SY	0.955 3	0.977 9	0.977 8		0.019 1 (127.7)	0.035 3 (219.1)	0.041 0 (161.2)
MW	0.954 1	0.982 3	0.979 9	0.983 8		0.028 4 (174.7)	0.032 0 (136.2)
ML	0.941 8	0.973 3	0.975 6	0.968 3	0.974 5		0.016 3 (58.2)
XD	0.936 2	0.968 4	0.970 9	0.962 8	0.971 0	0.986 7	

Data below and above diagonal indicate genetic identity and genetic distance, respectively. The number in parentheses is geographical distance (km) between populations.

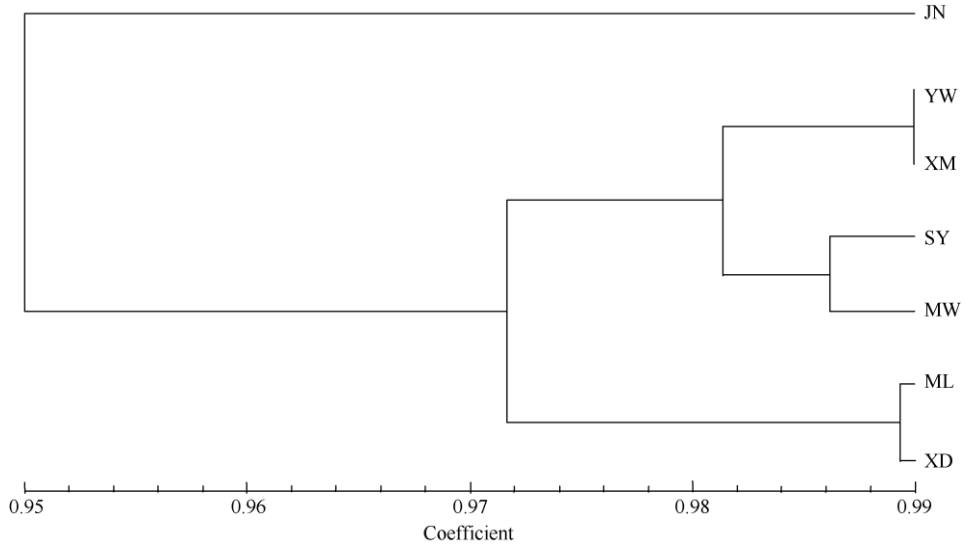

Fig. 3 UPGMA tree of *Scleropyrum wallichianum* populations

3 Discussions

3.1 Genetic diversity

According to phenotype data of seeds, Wu et al.[6] reported that there were big phenotype variations (93.72%) within populations of *S. wallichianum*, and small variations among seven populations (6.28%). Based on the content of fatty acid of *S. wallichianum*, Wu et al.[7] considered that the variation within populations was larger than that among populations.

In our study, similar results was obtained on the basis of the AFLP data, which revealed that 78.3% of the total variations distributed within populations, and 22.7% among populations.

The high genetic diversity of *S. wallichianum* may be attributed to its biological characteristics and geographical distribution. According to the record of *Floral of China*, *Scleropyrum wallichianum* mainly distributed in Guangxi, Hainan, Yunnan Provinces, and Indo-China Peninsula. Taking our sampling into consideration, these samples gathered in Yunnan represented the northern border of distribution area of the species, where historically complex tectonic events continually taken place. It is possible that these samples had sympatric distribution areas before the Yunnan-Guizhou Plateau uplift, causing low level of genetic differentiation between populations. In addition, the latitudes of *S. wallichianum* distributed range from 900~1 600 m, and grow in mixed forest habitats. Long-term adaptability to diverse environments was conducive to the accumulation and preservation of genetic variation. Even single and small distribution area was formed after geological uplifting, but the genetic variation speed among population was slower than that of within populations.

3.2 Genetic structure

In this study, a high level of genetic differentiation among population was detected with G_{ST}=0.191, and φ_{PT}=0.135. Among the many factors influencing inter-population genetic differentiations, gene flow was the most obvious. Population experiencing high gene flow tended to have lower genetic differentiation than that of populations with limited gene flow. In this study, *Nm* was estimated to be 2.141, which suggested the existence of a strong gene exchange among these seven populations. The proliferation and migration of individuals or propagules was associated with gene flow among different populations[17]. According to our observation in the wild field, *Scleropyrum wallichianum* relies mainly on animals for seed dispersal, which provided opportunities for genetic recombination between populations. For pollen dispersal, because no investigation and studies recorded for this species, we don't know whether or not the pollen dispersal increase the gene flow between populations.

3.3 Genetic relationships

According to the results of cluster analysis based on genetic similarity, most germplasms from the same population mixed with other clusters (Fig. 2), which suggested wide genetic variability existed in these seven populations. The habitat of all populations was hillside and ridge. Similar environment may be reasonable for the cross-distribution of these germplasms. At population level, population YW and XM from Mengla County was clustered at first, they and populations SY and MW then clustered into one group, which had close genetic relationship with the cluster of populations ML and XD. Population JN, however, was only distantly related to the other populations. Population ML and SY had the farthest geographical distance (219.1 km) (Table 2 and Fig. 3), but their genetic distance was not the largest. This suggested that genetic relationship was not strictly related to geographic distance among these seven populations. Furthermore, there was no significant correlation between genetic distance and geographical distance (r=0.0323, P=0.5820) for these populations as a whole.

4 Introduction and conservation strategy

As a potential resource for extracting natural acetylenic acid, and the material of nut foods, *Scleropyrum wallichianum* should be paid more attention, especially to the natural resource exploring and conservation. Therefore, our results in this paper could provide necessary information for selecting excellent individuals and germplasm resources with abundant variation. Eleven germplasms, such as JN14, MW63, JN13, YW18, XD75, YW25, MW62, YW17, SY49, JN8 and ML78, were selected with high polymorphism of specific loci. These germplasms, accounting for 20.53% of total specific bands, should be priority in protection and attention.

For commercial resource, the seeds of *S. walli-*

chianum were valuable, so, the fruitful germplasms should be introduced. But for species diversity, the germplasms with large genetic variation should be protected. From this study and based on the present situation of *S. wallichianum* distribution, we consequently proposed the following conservation strategies. Partly germplasms can be protected *in situ*, because this species mainly grow around the village, accompanying with farming plant, and the seeds can provide a fine food for local people. For *ex situ* conservation, the eleven germplasms above-mentioned can be introduced in order to increase genetic diversity. Additionally, these eleven germplasms should be well maintain the sustainable development of existing genetic diversity of *S. wallichianum.*

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