

# RAPD analysis for genetic diversity of germplasm resources of *Strobilanthes*\*

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## ABSTRACT

The 18 samples representing 18 populations of *Strobilanthes cusia* (Nees) O. Ktze in Fujian Province of China were analyzed with RAPD markers. Eleven primers were used, a total of 106 bands were scored and 88 of them were polymorphic. The percentage of polymorphic loci was 77.36%, Nei's gene diversity was 0.2420, and Shannon's index was 0.3700. The 18 populations were classified into 2 groups based on the RAPD data by the method of hierarchical cluster analysis. Most of the populations from Fujian were clustered into a group, other populations were clustered into the other group. There was a high level of genetic diversity among the populations, and the genetic differentiation was obvious among the populations from Fujian.

**Keywords:** *Strobilanthes cusia* (Nees) O. Ktze; RAPD; Germplasm Resources; Genetic Diversity

## 1. INTRODUCTION

*Strobilanthes cusia* (Nees) (O. Ktze) belong to Acanthaceae. It is distributed in southern China, including Zhejiang, Fujian, Hunan, Guangxi, Guangdong, Sichuan, Guizhou, Yunnan etc. [1]. It is an original plant of Indigo, an Chinese medicine product. The indigo produced in Fujian has the best quality, and famous for "jian indigo" [2]. Therefore, Fujian is the origin place of this famous local drug.

There is a long history of cultivation and rich wild resources of *Strobilanthes cusia* (Nees) O. Ktze in Fujian. However, as the destruction of natural environment, the distribution area of wild resources has decreased, whereas

the market demand for *Strobilanthes cusia* (Nees) O. Ktze has significantly increased with the increase in drug application of Indigo. No studies on the resources of *Strobilanthes cusia* (Nees) O. Ktze in Fujian has been reported. It is important to develop the artificial cultivation and strengthen the investigation, collection and protection of wild species resources of *Strobilanthes cusia* (Nees) O. Ktze. Although some morphological characters of the *Strobilanthes cusia* (Nees) O. Ktze vary greatly, they are easily affected by environmental factors. Hence, comparison only based on the morphological characters will not be able to reveal the diversity of germplasm resources of *Strobilanthes cusia* (Nees) O. Ktze effectively and scientifically. Using molecular markers to analyze genetic diversity, genetic characteristics and genetic relationship among different germplasms will provide theoretical foundation for the introduction, resource protection and breeding of *Strobilanthes cusia* (Nees) O. Ktze.

## 2. MATERIALS AND METHODS

### 2.1. Plant Materials

Eighteen samples each representing an individual population of *Strobilanthes cusia* (Nees) O. Ktze (Table 1) were collected from 25 sampling locations in 21 counties of Fujian and some other nearby provinces. The samples were identified by professor Wei Dao-zhi and preserved in the herbarium of Fujian Agriculture and Forestry University.

### 2.2. DNA Extraction

The CTAB method [3] was used to extract total DNA. Fresh leaves should be used because *Strobilanthes cusia* (Nees) O. Ktze are rich of phenols and other secondary metabolites including indigo, which can be oxidized and bound with DNA easily so as to affect DNA extraction.

### 2.3. Selection of Primers

Eleven primers that could yield clear, stable and reliable PCR products with abundant polymorphisms were

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**Table 1.** The collecting locations of *Strobilanthes cusia* (Nees) *O. Ktze* samples.

Sample Number	collecting location
QY	QingYun maintain, Yongtai county, Fujian province
CB	Chibi, Yongtai county, Fujian province
XL	Zijin maintain, Xinluo district, Longyan city, Fujian province
ZP	Shimen village, Pingguan town, Zhangpingcounty, Longyan city, Fujian province
XK	Forestry station, Gulai village, Xianyou county, Fujian province
SF	Shufeng town, Xianyou county, Fujian province
BT	Wudun village, Bongtuo town, Xianyou county, Fujian Province
LT	Letu rainforest, Nanjin county, Fujian Province
CC	Xia maintain village, Chuanchang town, Nanjin county, Fujian Province
NP	Mangdang maintain natural reserve, Nanping city, Fujian Province
MH	Flag maintain, Minhou county, Fujian Province
SL	Forest park, Fuzhou, Fujian Province
ZQ	Dinghu maintain, Zhaoqing, Guangdong Province
GZ	Guangzhou city, Guangdong Province
CZ	Tiandeng county, Congzuo city, Guangxi Province
NN	Nanning city, Guangxi Province
GY	Guiyang city, Guizhou
SC	Yibin city, Sichuan

selected from 100 random primers (Sangon products; **Table 2**).

## 2.4. PCR Amplification and Detection of the Products

The PCR amplification was carried out in MyCycler PCR. The reaction system volume was 20  $\mu$ L, containing 10  $\times$  PCR buffer 2.0  $\mu$ L, dNTP mixture (each 2.5  $\text{mmol}\cdot\text{L}^{-1}$ ) 2.4  $\mu$ L, 20  $\text{mmol}\cdot\text{L}^{-1}$  primers 0.4  $\mu$ L,  $\text{Mg}^{2+}$  0.4  $\mu$ L DNA template 2  $\mu$ L (10  $\text{ng}\cdot\mu\text{L}^{-1}$ ) and Taq (5  $\text{U}\cdot\mu\text{L}^{-1}$ ) 0.2  $\mu$ L, DDW 12.6  $\mu$ L. The PCR program was: fore- denaturalization at 95°C for 5 min; 38 cycles of degeneration at 94°C for 45 s, renaturation at 38°C for 1min, extension at 72°C for 2 min; and final extension at 72°C for 5 min. The PCR products were separated by electrophoresis on 1.5% agarose gel and visualized by ultraviolet.

**Table 2.** Primers selected for the experiment.

Primer	Sequence	Primer	Sequence
S2	TGATCCCTGG	S174	TGACGGCGGT
S5	TGCGCCCTTC	S364	CCGCCAAAC
S7	GGTGACGCAG	S367	AGCGAGCAAG
S64	CCGCATCTAC	S368	GAACACTGGG
S65	GATGACCGCC	S369	CCCTACCGAC
S67	GTCCCGACGA		

## 2.5. Recording and Analysis of the Datas

PCR bands were recorded as 1 for presence and 0 for absence. The computer program POPGENE32 was used to analyze the genetic parameters of the populations, calculating percentage polymorphic bands (PPB), number of alleles per locus (Ao), effective number of alleles per locus (Ae), Nei's gene diversity (H), Shannon's information index (I), Nei's genetic identity (J), genetic distance (D). Cluster analysis was performed using the UPGMA method. The phylogenetic tree among the samples was established by calculating the continental distance with SPSS 13.0 analysis software. The between groups linkage method was applied to the cluster analysis.

## 3. RESULTS

### 3.1. Genetic Diversity of *Strobilanthes cusia* (Nees) *O. Ktze*

The PCR analysis on the 18 DNA samples of *Strobilanthes cusia* (Nees) *O. Ktze* with 11 primers detected 106 bands ranging 200 - 3000 bp (**Figures 1 and 2**), of which 88 were polymorphic. Based on these data, it could be calculated that the PPB = 77.36%, AO = 1.7736, Ae = 1.4025, H = 0.2420, and I = 0.3700. Among the 12 samples from Fujian, the PPB = 72.28%, Ao = 1.7228, Ae = 1.4060, H = 0.241, I = 0.3654. These results indicated that abundant genetic diversity existed among different *Strobilanthes cusia* (Nees) *O. Ktze* populations.

### 3.2. Genetic Similarity and Genetic Distances between *Strobilanthes cusia* (Nees) *O. Ktze* Populations

The genetic distances and genetic similarity between the *Strobilanthes cusia* (Nees) *O. Ktze* populations were calculated according to the Nei method (**Table 3**). The results indicated that the genetic similarity between 18 populations was in range of 0.6321 - 0.8868. The populations of GY and BT had the maximum genetic similarity between them, while QY and CZ had the minimum. In contrast, the genetic distance between QY and CZ was

**Table 3.** Genetic similarities and genetic distances between *Srobilanthes cusia* (Nees) *O. Ktze* populations.

Populations	QY	XL	XK	MH	LT	CC	SL	SF	GY	BT	CB	NP	ZP	SC	CZ	NN	GZ	ZQ
QY	****	0.745	0.764	0.745	0.793	0.764	0.726	0.755	0.783	0.708	0.689	0.745	0.698	0.670	0.632	0.689	0.689	0.689
XL	0.294	****	0.736	0.698	0.821	0.811	0.698	0.726	0.774	0.698	0.679	0.755	0.745	0.679	0.642	0.679	0.679	0.698
XK	0.269	0.307	****	0.811	0.764	0.736	0.793	0.764	0.811	0.774	0.679	0.774	0.726	0.698	0.717	0.736	0.717	0.698
MH	0.294	0.359	0.209	****	0.783	0.660	0.811	0.726	0.755	0.717	0.698	0.717	0.708	0.660	0.660	0.717	0.717	0.717
LT	0.233	0.198	0.269	0.245	****	0.783	0.802	0.736	0.764	0.689	0.689	0.745	0.755	0.651	0.670	0.670	0.632	0.651
CC	0.269	0.209	0.307	0.415	0.245	****	0.698	0.802	0.793	0.717	0.717	0.793	0.708	0.698	0.660	0.698	0.660	0.679
SL	0.320	0.359	0.233	0.209	0.221	0.359	****	0.783	0.755	0.736	0.679	0.717	0.726	0.642	0.736	0.698	0.698	0.698
SF	0.281	0.320	0.269	0.320	0.307	0.221	0.245	****	0.859	0.821	0.764	0.802	0.717	0.689	0.726	0.726	0.708	0.726
GY	0.245	0.257	0.209	0.281	0.269	0.233	0.281	0.153	****	0.887	0.793	0.868	0.802	0.774	0.736	0.811	0.793	0.793
BT	0.346	0.359	0.257	0.333	0.373	0.333	0.307	0.198	0.120	****	0.811	0.849	0.783	0.793	0.793	0.793	0.830	0.811
CB	0.373	0.387	0.387	0.359	0.373	0.333	0.387	0.269	0.233	0.209	****	0.849	0.783	0.793	0.717	0.755	0.774	0.793
NP	0.294	0.281	0.257	0.333	0.294	0.233	0.333	0.221	0.142	0.164	0.164	****	0.859	0.830	0.755	0.774	0.811	0.793
ZP	0.359	0.294	0.320	0.346	0.281	0.346	0.320	0.333	0.221	0.245	0.245	0.153	****	0.802	0.745	0.764	0.764	0.745
SC	0.401	0.387	0.359	0.415	0.429	0.359	0.444	0.373	0.257	0.233	0.233	0.186	0.221	****	0.698	0.698	0.717	0.793
CZ	0.459	0.444	0.333	0.415	0.401	0.415	0.307	0.320	0.307	0.233	0.333	0.281	0.294	0.359	****	0.830	0.755	0.736
NN	0.373	0.387	0.307	0.333	0.401	0.359	0.359	0.320	0.209	0.233	0.281	0.257	0.269	0.359	0.186	****	0.830	0.755
GZ	0.373	0.387	0.333	0.333	0.459	0.415	0.359	0.346	0.233	0.186	0.257	0.209	0.269	0.333	0.281	0.186	****	0.849
ZQ	0.373	0.359	0.359	0.333	0.429	0.387	0.359	0.320	0.233	0.209	0.233	0.233	0.294	0.233	0.307	0.281	0.164	****

the maximum (0.4587), while that between GY and BT was the minimum (0.1201). These results suggest that there is sufficient genetic differentiation among the *Strobilanthes cusia* (Nees) O. Ktze's populations.

### 3.3. Cluster Analysis

The clustering analysis (Figure 3) also showed that BT and GY had the highest similarity and were classified into a group earliest. In addition, ZP was quite similar to NP and they were classified into a group.

GZ and ZQ had a higher similarity and were classified into a group. CZ and NN were in group b. The 12 populations from Fujian could be classified into five classes, SF and BT were grouped as class A, both of which belong to Xianyou's populations and have near geographical locations. ZP, NP and CB were grouped into class B.

XK, MH and SL were grouped as class C. They are all located in Fujian and have near geographic positions. LT, CC and XL were classified as class D. They are all located near geographically in the south area of Fujian province. QY was classified as a class E itself.

The above results indicate that the differentiation among populations is obvious, though the sampling was intensive from Fujian province. In addition, GY and SC were grouped into class A and B respectively. Finally, Class C, D and E of Fujian are grouped into class I; other small classes were grouped into class II. There were two major categories for all the samples.

It can be seen from the phylogenetic tree that most of the populations were classified into groups in consistency with their geographical locations, such as classes B, C and D from Fujian province. However, inconsistency

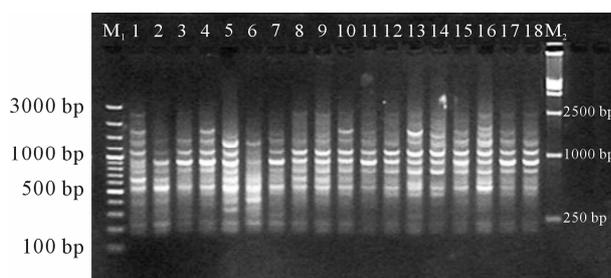


Figure 1. PCR results of primer S56.

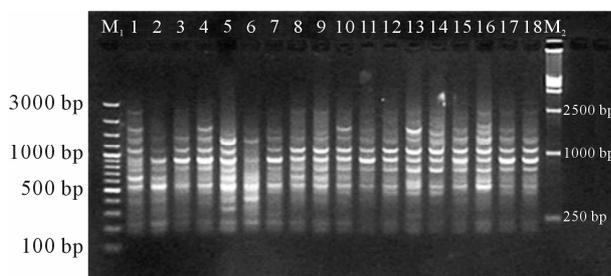


Figure 2. PCR results of primer S3677.

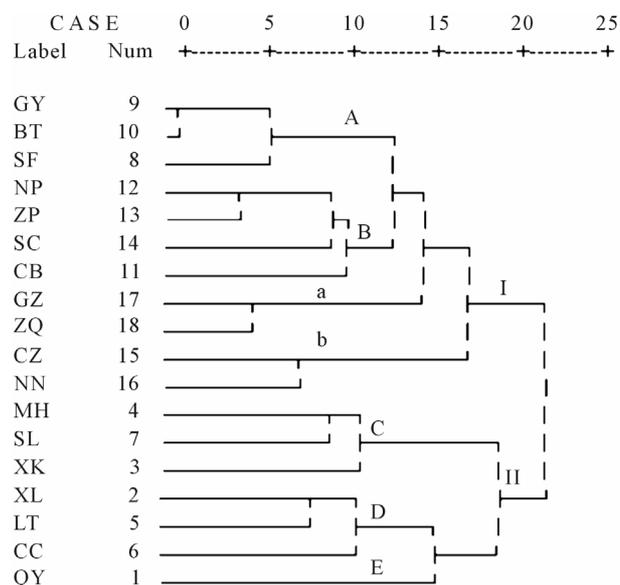


Figure 3. Phylogenetic tree of the populations.

also existed between the genetic distance and the geographical distance. For example, among the three populations from Xianyou (XM, SF, BT), only two were grouped into a class, suggesting that there might be two species in that production place. Although Guizhou and Sichuan are geographically far from Fujian, the populations from them were classified into the same class with those from Fujian, respectively. It was explained that geographical locations and environmental factors are very important for the formation of resources class. Although they are from different locations, their own genetic characteristics are relatively stable.

## 4. DISCUSSION

*Strobilanthes cusia* (Nees) O. Ktze contains a lot of carbohydrates, phenols and other secondary metabolites. These substances can severely disturb DNA extraction.

The indigo is rich in *Strobilanthes cusia* (Nees) O. Ktze's leaves. It is vulnerable to oxidation, and can bind with DNA easily, resulting in the damage of DNA. Leaves may become black when indigo is oxidized, which would lead to DNA contraction, looking yellow or light brown. Therefore, the leaves used for DNA extraction should be fresh and saved in liquid nitrogen rapidly.

The geographical location and environmental factors are important external factors that make resources heterogeneous. This is morphologically obvious among different populations and between wild and cultivated species. However, some populations with far genetic distances exhibit little morphological difference. Hence, morphological traits are not accurate indicators for determining the genetic relationships among different populations of *Strobilanthes cusia* (Nees) O. Ktze. In-

stead, molecular markers can reveal the genetic relationships among accessions more effectively and accurately.

The results of this study indicate that *Strobilanthes cusia* (Nees) O. Ktze has a strong adaptive capacity to environment and thus exhibits high genetic diversity. The natural populations of *Strobilanthes cusia* (Nees) O. Ktze in Fujian are concentrated in Fuzhou, Putian and Zhangzhou. These districts are abundant of wild communities that are large and grow well. They were important germplasm resources for breeding. In recent years, along with the deterioration of their habitat environment and increasing collection in disorder, the wild germplasm resources of *Strobilanthes cusia* (Nees) O. Ktze are decreasing seriously. Therefore, protection of the genetic resources of *Strobilanthes cusia* (Nees) O. Ktze in Fujian has become urgent.

## 5. ACKNOWLEDGEMENTS

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