



🔒 Assessment of genetic diversity in *Emmenopterys henryi*, a rare and endangered plant, based on SRAP and SSR fluorescent markers

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Yanli Niu¹, Yansong Peng¹, Wenxing Chen², Xuaihuai Zhan¹, Sadaruddin Chachar³, Pingxian Zhang², Saixia Zhou¹

¹Chinese Academy of Science, Lushan Botanical Garden, Jiangxi, 332900;

²Key Lab of Horticultural Plant Biology, Huazhong Agricultural University, China;

³Chinese Academy of Agricultural Sciences, Biotechnology Research Institute, Beijing 100081



Yanli Niu

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Abstract

SSR and SRAP markers were applied to assess the genetic diversity and structure of four populations of sixty-four samples of *Emmenopterys henryi*. 12 pairs of SRAP primers generated 86 bands, and polymorphism rate was 88.4%. Higher values of genetic diversity parameters ($h = 0.3195$, $I = 0.4757$, $PPB = 69.377\%$, at the species level; $h = 0.2632$, $I = 0.3868$, $PPB = 89.53\%$, at the population level) demonstrated that relatively higher level of genetic diversity. A total of 150 alleles were detected using 10 pairs of SSR primer. The number of alleles per locus ranged from 7 to 24, with an average of 15 alleles of each locus. The genetic diversity parameters ($h = 0.091$, $I = 0.176$, $PPB = 99.33\%$, at the species level; $h = 0.0829$, $I = 0.1447$, $PPB = 50.34\%$, at the population level) were obtained. Population genetic differentiation of *E. henryi* (SRAP, $G_{ST} = 0.1936$; SSR, $G_{ST} = 0.0944$) was higher level compared to other species. AMOVA revealed that genetic variation mainly existed within populations. For SRAP markers, UPGMA dendrogram and PCA cluster analysis separate HS and DWS populations from other populations. But for SSR markers, UPGMA dendrogram and PCA cluster analysis divided 4 populations into 3 groups but was not obvious, and indicated the extent of admixture within some samples. It would seem the extant genetic pattern of *E. henryi* was might mainly attributed to its long evolutionary history, wide-ranging distribution, outcross mating system and human activities. According to our results, SRAP markers was more effective in evaluation genetic diversity of *E. henryi*.

Attachments

									
<u>Table1.pdf</u>	<u>Table2.pdf</u>	<u>Table3.pdf</u>	<u>Table4.pdf</u>	<u>Table5.pdf</u>	<u>Figure1.pdf</u>	<u>Figure2.pdf</u>	<u>Figure3A.pdf</u>	<u>Figure3B.pdf</u>	<u>Figure4.pdf</u>
75KB	79KB	25KB	25KB	44KB	118KB	281KB	270KB	292KB	159KB

Materials

1 Materials and methods

1.1 Plant materials

Sixty-four plants were randomly collected from 4 *E. henryi* populations from different regions, including National Nature Reserve and distribution where habitat was destroyed severely in Jiangxi Province and used for genetic diversity studies. Up to twenty plants, that were at least 100 meters distant from each other were selected randomly. Population locations and details of specimens studied are given in Fig.1 and Table.1. Voucher specimens have been deposited in the herbarium of Lushan Botanical Garden.

1.2 Extraction of genomic DNA

Genomic DNA was extracted from silica-dried leaves using the CTAB method with some modifications [1]. Concentration and quality of DNA were verified using a spectrophotometer (Nano Drop 2000, Thermo Scientific). Isolated DNA was diluted to 20 ng/μl and subsequently stored at -20°C. Table 2 shows the primer combinations and sequences.

1.3 PCR reaction

The optimal SRAP-PCR amplifications were carried out in 20 μl reaction mixtures containing 2 μL template DNA (20ng/μl), 10 uL Master MIX (Blue), 0.3 uL forward primers (0.3 μmol/L), 0.3 uL reverse primers (0.3 μmol/L), 0.2 uL *Taq* DNA polymerase (1U) and 7.2 uL sterile deionized water. The amplification program was as follows: initial denaturation at 94°C for 3 min, 5 cycles of denaturation at 94°C for 40 s, renaturation at 35°C for 40 s, 1 min extension at 72°C, followed by 36 cycles of denaturation at 94°C for 40 s, renaturation at 50°C for 40 s, 1 min extension at 72°C and a final extension at 72°C for 8 min 30 s. The amplified products were visualized on 1.5% agarose gel using GoldView staining and imaged with a gel imaging system.

The optimal SSR-PCR amplifications were carried out in 20 μl reaction mixtures containing 2 μL template DNA (20ng/μl), 0.3 uL forward primers (20 μmol/L), 0.3 uL reverse primers (20 μmol/L), 0.2 uL *Taq* DNA polymerase (1U) and 14.8 uL sterile deionized water. The amplification program was as follows: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, renaturation at 54°C for 35s, 40 s extension at 72°C and a final extension at 72°C for 3 min. PCR products are diluted by 10 times, mixed with the internal size standard ABI, run on capillary electrophoresis with 3730XL Sequencer and analyzed using Genemarker .

1.4 Data analysis

Each amplified band was scored with a binary code based on the presence (1) or absence (0) of a band. Various genetic diversity estimates such as the percentage of polymorphic loci (*PPB*), number of polymorphic loci (n_p), effective number of alleles (*Ne*), observed number of alleles (*Na*), Shannon's Information index (*I*) and Nei's gene diversity (*h*) were calculated using POPGENE version 1.32.

Genetic similarity was calculated with NTSYS-PC 2.10 software. The samples were grouped by cluster analysis using the unweighted-pair group method with arithmetic averages (UPGMA) [2]. Genetic relationships among the genotypes were analyzed by principal component analysis (PCA) in MVSP [3]. STRUCTURE [4] software was used to infer population structure. Ten independent runs were made with values of K set from 1 to 6, with three iterations for each value of K. The length of the burn-in period was set at 10,000 and the number of Markov chain Monte Carlo (MCMC) repeats after burn-in was set at 100,000. Evanno's method [5] was implemented with STRUCTURE HARVESTER, developed by Earl and Vonholdt [6], to determine the value of estimated Ln probability of data, Ln P(K) and to get the best fit value of K for the data. The significant differences between groups and samples were tested by analysis of molecular variance in GenAlEx 6.5 [7].

