

# 广东省野百合天然居群的随机扩增多态性 DNA (RAPD) 分析

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**摘要:** 本研究选择了中国广东省 7 个有代表性的山区作为野百合 (*Lilium brownii* F. E. Brown ex Mieliez) 天然居群的采样点, 对采集到的 199 份样品进行了随机扩增多态性 DNA (RAPD) 分析, 以建立野百合天然居群 RAPD 分析方法体系以便后续研究, 并了解广东省野百合天然居群多态性情况和居群内外个体及野百合及变种百合样品的聚类情况。以新鲜叶片、硅胶干燥叶片及鳞片为材料分别提取 DNA, 从 273 条 10 聚寡核苷酸随机引物中筛选出 20 条随机引物, 对 7 个居群共 199 个野百合及百合样品进行 RAPD 扩增。共检测到 433 条 RAPD 谱带, 其中多态性条带为 430 条, 所有个体的多态性条带百分率 (PPB) 为 99.31%, 各居群的平均多态位点比率为 60.67%。通过对 199 个个体的 RAPD 数据的 UPGMA 聚类分析, 获得了聚类图。199 个样品用三种方法都成功提取出质量较高的 DNA, 并能扩增出清晰、明亮、稳定的 RAPD 条带。在聚类图遗传相似系数为 0.68 处发现同一居群内的所有百合个体和野百合个体在聚类图中表现了明显的分离, 这是原变种野百合及变种百合的区别在分子水平上很好的反映。

**关键词:** *Lilium brownii*; 野百合; 百合; RAPD; 聚类分析

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## Random Amplified Polymorphic DNA (RAPD) Analysis of Natural *Lilium brownii* from Guangdong, China

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**Abstract:** *Lilium brownii* F. E. Brown ex Mieliez, Brown lily, belongs to *Lilium* of *Liliaeae* in plant taxonomy, is a endemic species and one of commonly-used cross breeding parents of *Lilium* in China. *L. brownii* F. E. Brown ex Mieliez var. *viridulum* Baker, Greenish lily, a variety of *Lilium brownii*, is also a well known medicinal and edible plant. Wild germplasm resources are the basic materials for genetic research and plant breeding. However, due to *Lilium brownii* is scattered in the wild and its collection is generally only in flowering phase, it is a great difficulty for its scientific research. In the year before, researches on *Lilium brownii* especially molecular biology researches are still lacking in Guangdong province. In this study, total 199 *Lilium brownii* samples of 7 natural populations collected from representative mountain areas in Guangdong China were analyzed by RAPD markers, so as to provide good foundation for further natural germplasm resources and related studies of *Lilium brownii* populations, and provide good evidence of the discrimination of Brown lily and Greenish lily at molecular level. DNA from all samples was extracted from leaves and scales, which amplified clear, bright and steady RAPD bands. 20 primers were screened from 273 random primers, and a total 433 DNA bands were amplified, 430 (99.3%) of which were polymorphic, the percentage of polymorphic bands (PPB) at the population level was 60.67% on average. The unweighted pair group method arithmetic average (UPGMA) cluster analysis of the RAPD data from 199 samples showed that all Brown lily samples and Greenish lily samples in the same population separated obviously at

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the value of 0.68 of genetic similarity coefficient, which was a good evidence of the discrimination of Brown lily and Greenish lily at molecular level.

**Key words:** *Lilium brownii*; Brown lily; Greenish lily; RAPD; Cluster Analysis

## 1 Introduction

*Lilium* is a great genus assembling medicinal, edible and ornamental value, which includes abundance species and cultivars. China has a long history of lilies' cultivation and application and known as one of the lily origin center. *Lilium brownii* F. E. Brown ex Mieliez, Brown lily, belongs to *Lilium* of *Liliaeae* in plant taxonomy, is a endemic species and one of commonly-used cross breeding parents of *Lilium* in China<sup>[1-2]</sup>. *L. brownii* F. E. Brown ex Mieliez var. *viridulum* Baker, Greenish lily, a variety of *Lilium brownii*, is also a well known medicinal and edible plant. Wild germplasm resources are the basic materials for genetic research and plant breeding. However, due to *Lilium brownii* is scattered in the wild and its collection is generally only in flowering phase, it is a great difficulty for its scientific research. In the year before, the study on *L. brownii* focused mainly on few areas such as cytotaxonomy, tissue culture and cross breeding<sup>[3]</sup>. Recently, studies on wild populations of *Lilium* develop gradually<sup>[4-8]</sup>, while researches on *Lilium brownii* especially molecular biology researches are still lacking in Guangdong province, the southern breeding region of *Lilium*<sup>[9]</sup>, would not fulfill the requirement of exploitation and utilization of this wild plant resource.

Randomly amplified polymorphic DNA (RAPD) markers have proven to be a reliable method for determining genetic relationships among germplasm collections<sup>[10]</sup>. RAPD technique gained importance due to its simplicity, efficiency, relative ease to perform and nonrequirement of sequence information<sup>[11]</sup>. This relatively inexpensive molecular method has been widely used in applied and basic research in many different plant families<sup>[12-20]</sup>.

In this study, total 199 *Lilium brownii* samples of 7 natural populations collected from representative mountain areas in China were analyzed by RAPD markers, so as to provide good foundation for further natural germplasm resources and related studies of *Lilium brownii* populations, and provide good evidence of the

discrimination of Brown lily and Greenish lily at molecular level.

## 2 Materials and methods

### 2.1 Plant Material

A total of 199 individuals (160 Brown lily and 39 Greenish lily) were collected from Daqiao town Ruyuan County (pop R), Fenghuang Mountain in Chaozhou City (pop C), Maofeng Mountain in Guangzhou City (pop G), Dawu Mountain in Xinyi City (pop X), Lechang County (pop L), Wutong Mountain in Shenzhen City (pop S), and Nankun Mountain in Huizhou City (pop H) in Guangdong province, China. (Fig. 1, 2.), young leaves were collected and dried quickly with silica gel in sealed plastic bags in the field and then stored at  $-80^{\circ}\text{C}$ . The voucher specimens were

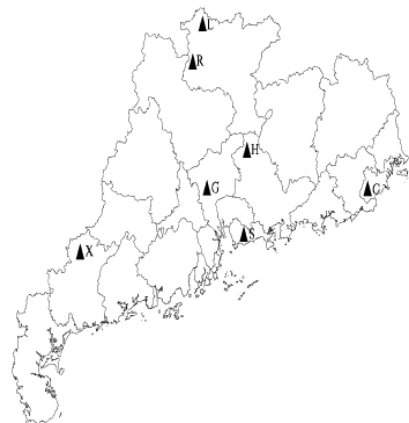


Fig. 1 Map showing the locations of the natural populations of *L. brownii* in Guangdong province, China sampled in this study



Fig. 2. *L. brownii* and its habitat

deposited at the herbarium of South China Agricultural University.

## 2.2 DNA Extraction and RAPD-PCR Amplification

Genomic DNA of all samples was isolated from the fresh leaves, silica gel dried leaves and scales DNA concentration and quality were determined in 0.8% agarose gels. Random 10-base primers (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd) which produced strong, clear and reproducible bands were screened for RAPD analysis. RAPD-PCR reactions were carried out in a 20  $\mu$ L reaction volume containing 20 ng template DNA, 2.0 mmol/L  $MgCl_2$ , 0.2 mmol/L of each dNTP (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd), 1.5 U *Taq* DNA polymerase (Sino-American Biotech Co., Ltd), and 0.3  $\mu$ mol/L primer. The RAPD-PCR amplifications were conducted in a PTC-100TM Peltier Thermal Cycler (MJ Research<sup>TM</sup> incorporated) and run through the following temperature profile: A hot start at 94 $^{\circ}C$  followed an initial denaturation of 5 min at 94 $^{\circ}C$ , this was followed by 35 cycles of 30 s at 94 $^{\circ}C$ , 50 s at 38 $^{\circ}C$ , and 1 min at 72 $^{\circ}C$ , and a final step of 10 min extension at 72 $^{\circ}C$ .

The amplification products were separated via electrophoresis, A 100 bp DNA Ladder Plus (Jingmei BioTech Co. Ltd) was used as a reference for sizing the fragments obtained. Staining was achieved with 5  $\mu$ L/100 mL goldview fluorescent dye (Beijing SBS Genetech Co. Ltd) added to each gel. They were then photographed and evaluated with the JD801 Gel Electrophoresis image acquisition and analysis system 3.3.1 (Jiangsu JEDA Science-Technology Development Co., Ltd)<sup>[21]</sup>.

## 2.3 Data analysis

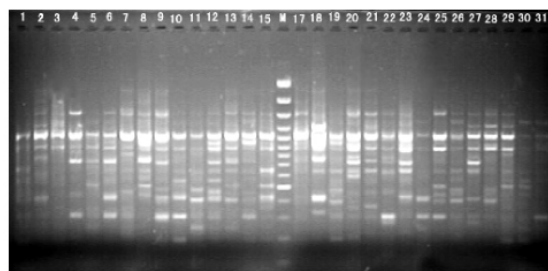
Since RAPD markers are dominant, it was assumed that each band represents the phenotype at a single biallelic locus<sup>[11]</sup>. Amplified fragments were scored as “1” for presence and “0” for absence of homologous bands, and a matrix of different RAPD phenotypes was established and used for statistical analysis. Only clear, unambiguous, consistent and reproducible bands between 350 and 2 500 bp were considered for data analysis, weak bands were excluded. After treated by DCFA2.0 software<sup>[22]</sup>, the binary data

matrix was analysed using the program POPGENE v 1.32<sup>[23]</sup>. Based on Nei's gene diversity, UPGMA dendrogram was drawn by NTSYSpc (v 2.10)<sup>[24]</sup>.

## 3 Result and Discussion

### 3.1 Result of RAPD Analysis

20 primers were screened from 273 random primers, which generated a total of 433 bands (loci) that ranged in size from 190 to 2 330 base pairs (Fig. 3) across all 199 individuals of the 7 populations of *L. brownii*. The primers yielded 14 to 29 bands, with an average of 21.7 bands per primer. Among the 433 bands, 430 (99.31%) were polymorphic. The percentage of polymorphic bands (PPB) at the population level was 60.67% on average.



M. Marker, 100 bp DNA Ladder Plus; 1–15 and 17–31. show RAPD pattern of samples from Ruyuan population using primer S1345.

Fig. 3 RAPD pattern using the template DNA of *Lilium brownie*

### 3.2 Cluster Analysis of All Individuals

An UPGMA dendrogram based on Jaccard's similarity coefficient between all individuals studied was shown in Fig. 4. At the value of 0.52 of genetic similarity coefficient, individuals from the same population were clearly clustered into one subgroup on the whole, which implied that genetic differentiation among populations was significant. The dendrogram also showed that there was high genetic variation within populations.

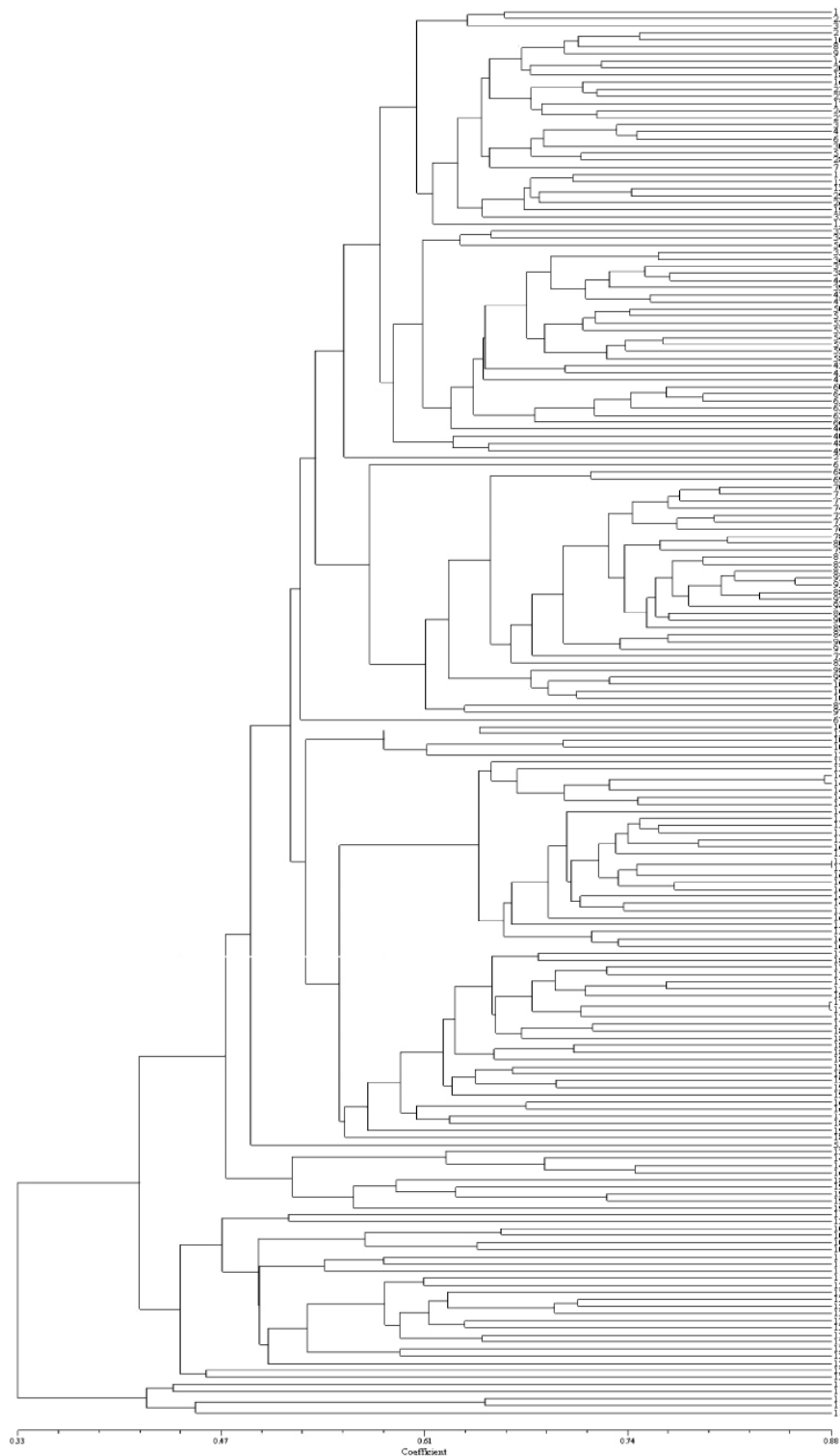
### 3.3 The Classification of Brown Lily and Greenish Lily

According to our field survey, Brown lily distributes widely all over Guangdong province, while Greenish lily is available only in the northern regions of Guangdong, so in the 7 populations studied, greenish lily exists only in pop R and pop L.

The unweighted pair group method arithmetic average (UPGMA) cluster analysis of the RAPD data

from 199 samples from 7 populations showed that all Brown lily samples and Greenish lily samples in the same population separated obviously at the value of

0.68 of genetic similarity coefficient (Fig. 4), which is a good evidence of the discrimination of brown lily and greenish lily at molecular level.



1-32. Denote samples from pop R; 33-59. Denote samples from pop C; 60-67. Denote samples from pop G; 68-96. Denote samples from pop X; 97-136. denote samples from pop L; 137-167. denote samples from pop S; 168-199. denote samples from pop H; 14, 16-18, 20, 22-24, 27, 32, 104-107, 110-123, 125-126, 128-136. Greenish lily; the other samples are Brown lily.

**Fig. 4** UPGMA dendrogram of 199 individuals of 7 *L. brownii* populations based on Jaccard's similarity coefficient

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### References:

- [1] 龙雅宜, 张金政. 百合属植物资源的保护与利用[J]. 植物资源与环境, 1998, 7(1): 40-44.
- [2] 夏宜平, 高晓辰. 试论百合等球根花卉的商品种球国产化问题[C]. //高俊平, 姜伟贤. 中国花卉科技进展-第二届全国花卉科技信息交流会论文集. 北京: 中国农业出版社, 2001: 216-223.
- [3] 江雪梅, 杨懋勋, 张应扬, 等. 野百合的研究概况[J]. 中国野生植物资源, 2006(4): 25-27.
- [4] 刘红美, 冷狐克勇, 方小波. 野百合试管鳞茎诱导与增殖的研究[J]. 安徽农业科学, 2008, 9(1): 18-20, 53.
- [5] 罗凤霞, 李雨, 沈向群, 等. 应用分子标记探讨一种野生百合的分类地位[J]. 沈阳农业大学学报, 2008, 39(6): 669-672.
- [6] 邵红, 宋小涛, 沈伟. 野百合的组织培养研究[J]. 佳木斯大学学报: 自然科学版, 2009, 27(2): 312-314, 320.
- [7] 荣立苹, 雷家军, 王志刚. 东北地区野生百合遗传多样性的 RAPD 分析[J]. 江苏农业学报, 2009, 25(4): 843-846.
- [8] 唐艳平, 刘秀群, 傅强, 等. 长江中游地区野生百合资源调查及利用前景[J]. 中国野生植物资源, 2010, 29(6): 18-22.
- [9] 吴学尉, 丁仁展, 屈云慧, 等. 现代百合品种发展趋势[J]. 北方园艺, 2007(2): 51-52.
- [10] Ruan C J, Qin P, Zheng J W, et al. Genetic relationships among some cultivars of sea buckthorn from China, Russia and Mongolia based on RAPD analysis[J]. Sci Hort, 2006, 101(2004): 417-426.
- [11] Williams J G K, Kubelik A R, Livak K J, et al. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers[J]. Nucleic Acids Res, 1990, 18: 6531-6535.
- [12] Gustafson D J, Gibson D J, Nickrent D L. Genetic diversity and competitive abilities of *Dalea purpurea* (Fabaceae) from remnant and restored grasslands[J]. Int J Plant Sci, 2002, 163: 979-990.
- [13] Gustafson D J, Gibson D J, Nickrent D L. Conservation genetics of two co-dominant grass species in an endangered grassland ecosystem[J]. J Appl Ecol, 2004, 41: 389-397.
- [14] Chen S L, Xia T, Chen S Y, et al. RAPD profiling in detecting genetic variation in endemic *Coelonema* (Brassicaceae) of Qinghai-Tibet Plateau of China[J]. Biochem Genet, 2005, 43(3): 189-201.
- [15] Zhao N X, Gao Y B, Wang J L, et al. Genetic Diversity and Population Differentiation of the Dominant Species *Stipa krylovii* in the Inner Mongolia Steppe[J]. Biochem Genet, 2006, 44(11): 513-526.
- [16] Shah A, Li D Z, Gao L M, et al. Genetic diversity within and among populations of the endangered species *Taxus fuana* (Taxaceae) from Pakistan and implications for its conservation[J]. Biochem Systematic. Ecol, 2007, 36(2008): 183-193.
- [17] Ye Y M, Zhang J W, Ning G G, et al. A comparative analysis of the genetic diversity between inbred lines of *Zinnia elegans* using morphological traits and RAPD and ISSR markers[J]. Sci Hort, 2008, 118(2008): 1-7.
- [18] An N, Guo H B, Ke W D. Genetic variation in rhizome lotus (*Nelumbo nucifera* Gaertn. ssp. *nucifera*) Germplasms from China assessed by RAPD markers[J]. Sci Agric Sin, 2010, 38(1): 31-39.
- [19] 陈邴俊, 马虹, 左开井, 等. 利用 RAPD 标记分析百合种质的遗传多样性[J]. 上海交通大学学报: 农业科学版, 2009, 27(5): 475-479.
- [20] 董巧珍, 周日宝, 刘湘丹, 等. 百合鳞叶 DNA 提取及 RAPD 分析[J]. 中南林业科技大学学报, 2010, 30(1): 48-53.
- [21] 黄永芳, 杨懋勋, 柳军, 等. 广东野百合 DNA 提取和 RAPD 条件的优化[J]. 热带亚热带植物学报, 2006, 14(3): 251-255.
- [22] 张富民, 葛颂. 群体遗传学研究中的数据处理方法 I. RAPD 数据的 AMOVA 分析[J]. 生物多样性, 2002, 10: 438-444.
- [23] Yeh F C, Yang R C, Boyle T. PopGene Version 1.32, Microsoft Windows Base Software for Population Genetic Analysis: A Quick User's Guide[M]. Alberta (Canada): Center for International Forestry Research, University of Alberta, 1999.
- [24] Rohlf F J. NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System, Version 1.80[M]. New York: Exeter Publishing, 1994.