

## 鱼腥草种质资源的 RAPD 分析

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**摘要:** 目的 分析鱼腥草种质资源在分子水平上的遗传多样性。方法 应用 RAPD 技术对 92 份鱼腥草种质资源进行检测。结果 34 个随机引物中有 32 个引物(94.1%)扩增产物具多态性。34 个引物共得到 200 条扩增 DNA 片段,其中 93.5% 的片段具多态性。每个多态性引物平均可扩增出 5.8 个多态性片段。峨眉蕺菜和蕺菜种内平均遗传相似系数(genetic similarity, GS)分别为 0.521 和 0.572,二者种间 GS 值为 0.517。峨眉蕺菜与蕺菜中染色体数目为 36 的细胞型间相似程度最高,其平均 GS 值达 0.530。栽培蕺菜类群比其野生类群遗传多样性相对较高。聚类分析表明,利用 RAPD 技术可将全部供试材料区分开,所有材料共划分为 14 类。其中,绝大多数(62 个)聚为一类,且根据 RAPD 遗传相似系数划分的类群同地理分布有一定关系。结论 (1) 鱼腥草种质资源在分子水平上确实存在较大遗传差异。(2) RAPD 标记可作为构建鱼腥草 DNA 指纹图谱的有效工具。(3) 鱼腥草药材道地性与环境因素有关,但更大程度上由其遗传因素所决定。

**关键词:** 鱼腥草; 蕺菜; 峨眉蕺菜; RAPD; 遗传多样性; DNA 指纹图谱; 道地性

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中药鱼腥草(*Herba Houttuyniae*)原植物为三白草科蕺菜属蕺菜 *Houttuynia cordata* Thunb.。具清热解毒、消痈排脓和利尿通淋等功能,为“既是药品,又是食品”的极具开发潜力的资源之一。据《中国植物志》和《四川植物志》等记载,蕺菜属仅蕺菜 1 种<sup>[1,2]</sup>。祝正银等<sup>[3]</sup>(2001)在四川峨眉山发现蕺菜属一新种峨眉蕺菜 *Houttuynia emiensis* ZY Zhu et SL Zhang,该种在峨眉、乐山等地俗称白侧耳根,也作蔬菜和鱼腥草药用。据作者初步调查,鱼腥草种质资源在形态特征、产量质量及抗病性等方面均有较大差异,且染色体数目不尽相同(另文报道)。但彼此间遗传背景、亲缘关系尚不清楚,故有必要运用新技术手段加强鱼腥草种质资源遗传多样性研究。RAPD 标记因其操作简便、DNA 用量少、PCR 引物无种属限制等特点,目前已广泛应用于人参<sup>[4]</sup>、西洋参<sup>[5]</sup>、当归<sup>[6]</sup>、石斛<sup>[7]</sup>、厚朴<sup>[8]</sup>、甘草<sup>[9]</sup>和苍术<sup>[10]</sup>等药用植物的 DNA 指纹图谱构建、真伪鉴定、亲缘关系、遗传多样性及药材道地性研究。本文利用 RAPD 技术对蕺菜和峨眉蕺菜进行遗传多样性分析,旨在探讨其种间、各材料间和不同细胞型蕺菜间遗传关系,以及鱼腥

草道地性产生的根本原因,为鱼腥草资源合理保护利用和新品种选育提供理论依据,也为鱼腥草药材 DNA 指纹图谱的构建奠定基础。

### 材 料 与 方 法

**材料** 试验材料采自四川、贵州及江苏等地不同地点或同一地点不同生境,共 92 份。其中峨眉蕺菜(EM)2 份,蕺菜 90 份。据细胞学观察,蕺菜不同材料的染色体数目不尽相同,本文按染色体数目将其划分为 12 个细胞型。A, B, C, D, E, F, G, H, I, J, K 和 L 分别代表染色体数目为 36, 54, 72, 80, 81, 82, 83, 84, 86, 88, 90 和 126 的不同细胞型。材料来源见表 1。

**DNA 的提取** 取鲜叶约 3 g 在液氮冷冻下研磨成粉末,置于 50 mL 离心管中,加入 15 mL 预热至 65 °C 的 2 × CTAB 提取缓冲液(100 mmol · L<sup>-1</sup> Tris-HCl pH 8.0, 10 mmol · L<sup>-1</sup> EDTA, 2% CTAB, 2% PVP, 0.2% β-巯基乙醇),65 °C 水浴保温 1 ~ 2 h,其间倒转离心管数次,取出离心管冷至室温。加入等体积氯仿-异戊醇(24:1),混匀,3 500 r · min<sup>-1</sup> 离心 12 min。取上清液加入 1/10 体积的 10% 的 CTAB,轻摇混匀,加等体积氯仿-异戊醇(24:1)混匀,3 500 r · min<sup>-1</sup> 离心 12 min,取上清液加等体积异丙醇,沉淀 DNA,用

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70 %乙醇冲洗 DNA 沉淀 2 ~ 3 次, 空气中干燥后用 TE 溶解备用。

**Table 1 The cytotypes and the sources of the germplasm resources of Herba Houttyniae**

| Cytotypes<br>(chromosome number) | Accessions No. | Locality                     | Habitat   |
|----------------------------------|----------------|------------------------------|---|
| EM (36)                          | W01-86         | Emei, Sichuan                | Botony Garden, cultivated                         |
|                                  | W01-1          | Jiufeng, Leshan, Sichuan     | River rapids, cultivated                          |
| A (36)                           | W01-4          | Wutongqiao, Leshan, Sichuan  | Thick growth of grass on the slope, cultivated    |
|                                  | W01-5          | Qingxi, Qianwei, Sichuan     | River rapids, cultivated                          |
|                                  | W01-9          | Yangjia, Jingyan, Sichuan    | Ridges between fields, growing wild               |
|                                  | W01-22         | Jinping, Yibin, Sichuan      | Thick growth of grass on the slope, growing wild  |
|                                  | W01-23         | Jingping, Yibin, Sichuan     | Ridges between fields, growing wild               |
|                                  | W01-24         | Yibin, Sichuan               | Thick growth of grass on the slope, growing wild  |
|                                  | W01-25         | Yibin, Sichuan               | Ridges between fields, growing wild               |
|                                  | W01-48         | Neijiang, Sichuan            | Thick growth of grass on the slope, growing wild  |
|                                  | W01-50         | Neijiang, Sichuan            | Thick growth of grass on the slope, growing wild  |
|                                  | B (54)         | W01-39                       | Hanlin, Pengshui, Chongqing                       |
| W01-43                           |                | Maerkang, Sichuan            | Vegetable fields under plastic shanty, cultivated |
| W01-97                           |                | Feixian, Lushan, Sichuan     | Tea plantation, cultivated                        |
| W01-105                          |                | Wangyu, Yaan, Sichuan        | Bamboo woods, growing wild                        |
| C (72)                           | W01-11         | Yangjia, Jingyan, Sichuan    | Bread-leaf woods, cultivated                      |
|                                  | W01-38         | Qianjiang, Chongqing         | Thick growth of grass on the slope, growing wild  |
|                                  | W01-52         | Youxian, Mianyang, Sichuan   | By drains, growing wild                           |
|                                  | W01-55         | Wulian, Jiange, Sichuan      | By drains, growing wild                           |
|                                  | W01-63         | Zhengyuan, Wangcang, Sichuan | Thick growth of grass on the slope, growing wild  |
|                                  | W01-81         | Taihusi, Yingjing, Sichuan   | Growing wild                                      |
| D (80)                           | W01-106        | Hejiang, Yaan, Sichuan       | Thick growth of grass on the slope, growing wild  |
|                                  | W01-16         | Richeng, Yibin, Sichuan      | Ridges between fields, growing wild               |
|                                  | W01-69         | Enyang, Bazhong, Sichuan     | Ridges between fields, growing wild               |
| E (81)                           | W01-80         | Huaishu, Mianyang, Sichuan   | By drains, growing wild                           |
|                                  | W01-2          | Jiufeng, Leshan, Sichuan     | River rapids, cultivated                          |
|                                  | W01-3          | Wutongqiao, Leshan, Sichuan  | Thick growth of grass on the slope, growing wild  |
|                                  | W01-6          | Jiujing, Qianwei, Sichuan    | Ridges between fields, growing wild               |
|                                  | W01-7          | Muxi, Muchuan, Sichuan       | Thick growth of grass on the slope, growing wild  |
|                                  | W01-8          | Maoqiao, Leshan, Sichuan     | Ridges between fields, growing wild               |
|                                  | W01-15         | Gongjing, Zigong, Sichuan    | River rapids, cultivated                          |
|                                  | W01-19         | Liunanba, Yibin, Sichuan     | Ridges between fields, growing wild               |
|                                  | W01-29         | Daguan, Nanxi, Sichuan       | Maize fields, cultivated                          |
|                                  | W01-30         | Daguan, Nanxi, Sichuan       | Citrus woods, cultivated                          |
|                                  | W01-31         | Hujia, Longchang, Sichuan    | Ridges between fields, growing wild               |
|                                  | W01-41         | Xindian, Mingshan, Sichuan   | Thick growth of grass on the slope, growing wild  |
|                                  | W01-42         | Wangu, Mingshan, Sichuan     | Thick growth of grass on the slope, growing wild  |
|                                  | W01-47         | Guza, Kangding, Sichuan      | Woods, growing wild                               |
|                                  | W01-54         | Wencang, Zitong, Sichuan     | Ridges between fields, growing wild               |
| F (82)                           | W01-71         | Mozixiang, Bazhong, Sichuan  | Ridges between fields, growing wild               |
|                                  | W01-72         | Jincheng, Yilong, Sichuan    | By drains, growing wild                           |
|                                  | W01-73         | Nanmu, Nanbu, Sichuan        | Woods on the slope, growing wild                  |
|                                  | W01-76         | Fuyi, Yanting, Sichuan       | By roads, growing wild                            |
|                                  | W01-77         | Fuyi, Yanting, Sichuan       | Thick growth of grass on the slope, growing wild  |
|                                  | W01-95         | Siyang, Lushan, Sichuan      | Vegetable fields, cultivated                      |
|                                  | W01-108        | Helong, Yaan, Sichuan        | By vegetable fields, growing wild                 |
|                                  | W01-114        | Nanjing, Jiangsu             | Botony Garden, Cultivated                         |
|                                  | W01-37         | Zhongdu, Youyang, Chongqing  | By drains, growing wild                           |
|                                  | W01-58         | Jianmen, Jiange, Sichuan     | Ridges between fields, growing wild               |
|                                  | W01-59         | Jianmen, Jiange, Sichuan     | By drains, growing wild                           |

Continued

| Cytotypes<br>(chromosome number) | Accessions No.         | Locality                         | Habitat  |
|----------------------------------|------------------------|----------------------------------|--|
| G (83)                           | W01-78                 | Fuyi, Yanting, Sichuan           | By river, growing wild                           |
|                                  | W01-96                 | Zhongfengsi, Emei, Sichuan       | Thick growth of grass on the slope, growing wild |
|                                  | W01-104                | Zhongjiang, Sichuan              | Vegetable fields, cultivated                     |
|                                  | W01-107                | Guanhua, Yaan, Sichuan           | Vegetable fields, cultivated                     |
|                                  | W01-18                 | Gaoxian, Sichuan                 | Ridges between fields, growing wild              |
|                                  | W01-62                 | Shangwu, Wangcang, Sichuan       | Ridges between fields, growing wild              |
|                                  | W01-66                 | Shahe, Nanjiang, Sichuan         | By rivers, growing wild                          |
| H (84)                           | W01-79                 | Lianghe, Yanting, Sichuan        | River rapids, growing wild                       |
|                                  | W01-45                 | Xuankou, Wenchuan, Sichuan       | Slope by roads, growing wild                     |
|                                  | W01-46                 | Ziping, Dujiangyan, Sichuan      | Thick growth of grass on the slope, growing wild |
|                                  | W01-51                 | Mojia, Mianyang, Sichuan         | Ridges between fields, growing wild              |
|                                  | W01-56                 | Liugou, Jiange, Sichuan          | By drains, growing wild                          |
|                                  | W01-68                 | Liangshui, Nanjiang, Sichuan     | Ridges between fields, growing wild              |
| I (86)                           | W01-92                 | Ziyang, Sichuan                  | Vegetable fields, growing wild                   |
|                                  | W01-13                 | Changshan, Rongxian, Sichuan     | Thick growth of grass on the slope, growing wild |
|                                  | W01-34                 | Junling, Xiushan, Chongqing      | Thick growth of grass on the slope, growing wild |
|                                  | W01-57                 | Hanyang, Jiange, Sichuan         | Thick growth of grass on the slope, growing wild |
|                                  | W01-64                 | Zhengyuan, Wangcang, Sichuan     | Woods, cultivated                                |
| J (88)                           | W01-98                 | Helong, Yaan, Sichuan            | Thick growth of grass on the slope, growing wild |
|                                  | W01-65                 | Sanjiang, Wangcang, Sichuan      | Thick growth of grass on the slope, growing wild |
|                                  | W01-83                 | Jialingqu, Nanchong, Sichuan     | Ridges between fields, growing wild              |
| K (90)                           | W01-99                 | Ziyang, Sichuan                  | Ridges between fields, growing wild              |
|                                  | W01-10                 | Yangjia, Jingyan, Sichuan        | Citrus woods, cultivated                         |
|                                  | W01-12                 | Changshan, Rongxian, Sichuan     | Ridges between fields, growing wild              |
|                                  | W01-14                 | Shuangshi, Rongxian, Sichuan     | Vegetable fields, cultivated                     |
|                                  | W01-33                 | Ganlong, Songtao, Guizhou        | Thick growth of grass on the slope, growing wild |
|                                  | W01-53                 | Youxian, Mianyang, Sichuan       | Vegetable fields, cultivated                     |
|                                  | W01-60                 | Dashi, Guangyuan, Sichuan        | Vegetable fields, growing wild                   |
|                                  | W01-61                 | Yuanba, Guangyuan, Sichuan       | Ridges between fields, growing wild              |
|                                  | W01-67                 | Shahe, Nanjiang, Sichuan         | Woods by rivers, growing wild                    |
|                                  | W01-70                 | Enyan, Bazhong, Sichuan          | Vegetable fields, cultivated                     |
|                                  | W01-74                 | Jianxin, Nanbu, Sichuan          | Ridges between fields, growing wild              |
|                                  | W01-75                 | Qianqiu, Nanbu, Sichuan          | Ridges between fields, growing wild              |
|                                  | W01-94                 | Wangyu, Yaan, Sichuan            | Thick growth of grass on the slope, growing wild |
|                                  | W01-100                | Qionglai, Sichuan                | Thick growth of grass on the slope, growing wild |
|                                  | W01-101                | yanchang, Yaan, Sichuan          | Tea plantation, growing wild                     |
|                                  | W01-103                | Qingchengshan, Sichuan           | Woods on the slope, growing wild                 |
|                                  | W01-109                | Shangli, Yaan, Sichuan           | Thick growth of grass on the slope, growing wild |
| W01-110                          | Xiali, Yaan, Sichuan   | On the slope, growing wild       |  |
| W01-112                          | Zhouhe, Yaan, Sichuan  | On the slope, growing wild       |  |
| W01-113                          | Yanqiao, Yaan, Sichuan | Woods on the slope, growing wild |  |
| L (126)                          | W01-102                | Zhouhe, Yaan, Sichuan            | Thick growth of grass on the slope, growing wild |

**RAPD反应** 用 Operon 公司生产的 A, B, H 等组的 60 多个十聚体随机引物对材料进行扩增筛选, 从中共筛选出谱带清晰的引物 34 个用于试验和统计分析, 反应在 PTC-220 PCR 仪上进行。反应总体积 25  $\mu\text{l}$ , 内含模板 DNA 20 ~ 50 ng, dNTPs 0.2  $\text{mmol} \cdot \text{L}^{-1}$ ,  $\text{MgCl}_2$  1.5  $\text{mmol} \cdot \text{L}^{-1}$ , 引物 0.2  $\mu\text{mol} \cdot \text{L}^{-1}$ ,  $1 \times \text{PCR buffer}$  (10  $\text{mmol} \cdot \text{L}^{-1}$  Tris-HCl pH 8.3, 5  $\text{mmol} \cdot \text{L}^{-1}$  KCl)。扩增前在 94  $^{\circ}\text{C}$  预变性 3 min, 每循环在 94  $^{\circ}\text{C}$

变性 1 min, 36  $^{\circ}\text{C}$  退火 1 min, 72  $^{\circ}\text{C}$  延伸 2 min, 共 50 个循环, 完成最后一个循环后, 在 72  $^{\circ}\text{C}$  保温 10 min。

扩增产物在 0.8% 琼脂糖凝胶上电泳分离。溴化乙锭染色, 凝胶成像仪上观察照像, 记录。

**数据处理** RAPD 扩增产物按条带有无分别赋值, 有带记为 1, 无带记为 0。计算供试材料间 Jaccard 遗传相似系数 (GS)<sup>[11]</sup>。利用 GS 按不加权重对群算术平均法 (unweighted pair-group method with

arithmetic avevage, UPGMA) 进行聚类。统计分析在 NTSYS-pc 软件系统下进行。

## 结 果

### 1 扩增片段多态性

RAPD 扩增结果表明, 34 个引物中有 32 个(占

94.1%) 引物扩增产物具多态性。图 1 为引物 OPH-20 的扩增结果。34 个引物共得到 200 条扩增 DNA 片段, 平均每个引物可获得 5.9 个 DNA 片段, 其中 93.5% 的片段具多态性。每个多态性引物能扩增出 2~11 个 DNA 片段, 平均可扩增出 5.8 个多态性片段, 表明鱼腥草种质资源多态性水平较高。

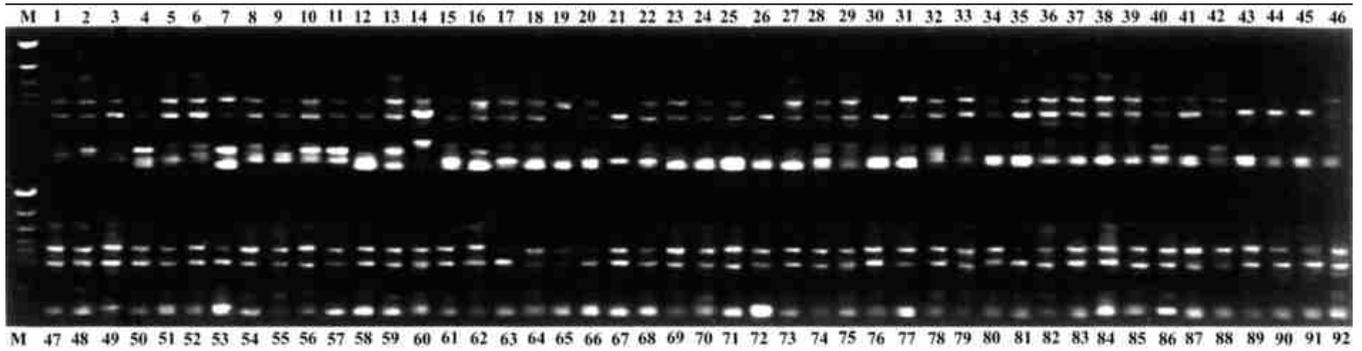


Figure 1 Amplified products of primer OPH-20 from 92 accessions

1 ~ 92 represent different accession in the sequence of Table 1. M represents PUC19/MSP DNA marker

### 2 遗传相似系数

#### 2.1 种内及类内平均遗传相似系数

峨眉蕺菜及 12 种蕺菜细胞型的类内平均 GS (genetic similarites) 值见表 2。从中可见, 峨眉蕺菜种内平均 GS 值为 0.521。蕺菜中, 以染色体数目为 82 的细胞型类内平均 GS 值(0.617)最大。染色体数目为 80 以上的细胞型类内 GS 平均值普遍较高, 变化范围在 0.572 ~ 0.617, 即其遗传多样性较小。染色

体数目相对较少的细胞型中, 除染色体数为 36 的细胞型类内平均 GS 值(0.596)较高外, 染色体数为 54 和 72 的细胞型类内平均 GS 均较低, 分别为 0.503 和 0.541, 表明其遗传多样性相对较丰富。所有蕺菜材料间 GS 平均值为 0.572, 变化范围 0.406 ~ 0.909(未列表)。其中, 以重庆彭水的 W01-39 和四川剑阁的 W01-59 间的 GS 最小, 四川井研的 W01-10 和荣县的 W01-12 间的 GS 值最大。

Table 2 The genetic similarities (GS) within and between *Houttuynia emeiensis* Z.Y. Zhu et S.L. Zhang(EM) and 12 cytotypes of *Houttuynia cordata* Thunb

| GS | EM    | A     | B     | C     | D     | E     | F     | G     | H     | I     | J     | K     | L |
|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---|
| EM | 0.521 |       |       |       |       |       |       |       |       |       |       |       |   |
| A  | 0.530 | 0.596 |       |       |       |       |       |       |       |       |       |       |   |
| B  | 0.522 | 0.539 | 0.503 |       |       |       |       |       |       |       |       |       |   |
| C  | 0.490 | 0.524 | 0.540 | 0.541 |       |       |       |       |       |       |       |       |   |
| D  | 0.528 | 0.539 | 0.574 | 0.561 | 0.576 |       |       |       |       |       |       |       |   |
| E  | 0.516 | 0.549 | 0.548 | 0.568 | 0.580 | 0.576 |       |       |       |       |       |       |   |
| F  | 0.524 | 0.538 | 0.559 | 0.580 | 0.610 | 0.595 | 0.617 |       |       |       |       |       |   |
| G  | 0.508 | 0.534 | 0.534 | 0.553 | 0.610 | 0.578 | 0.586 | 0.572 |       |       |       |       |   |
| H  | 0.504 | 0.528 | 0.541 | 0.565 | 0.591 | 0.583 | 0.598 | 0.574 | 0.566 |       |       |       |   |
| I  | 0.510 | 0.524 | 0.535 | 0.574 | 0.599 | 0.591 | 0.624 | 0.596 | 0.591 | 0.590 |       |       |   |
| J  | 0.529 | 0.559 | 0.560 | 0.572 | 0.625 | 0.599 | 0.624 | 0.608 | 0.601 | 0.615 | 0.613 |       |   |
| K  | 0.517 | 0.534 | 0.542 | 0.571 | 0.598 | 0.583 | 0.600 | 0.568 | 0.578 | 0.588 | 0.601 | 0.587 |   |
| L  | 0.524 | 0.493 | 0.551 | 0.564 | 0.541 | 0.589 | 0.633 | 0.534 | 0.579 | 0.589 | 0.560 | 0.586 | - |

Note: A, B, C, D, E, F, G, H, I, J, K and L represent different *Houttuynia cordata* Thunb. cytotype which has the chromosome number of 36, 54, 72, 80, 81, 82, 83, 84, 86, 88, 90 and 126, respectively

## 2.2 种间及类间遗传相似系数

从表 2 中还可看出,染色体数目为 36 的峨眉蕺菜与 12 种蕺菜细胞型间,以及与染色体数目为 72 的细胞型间遗传差异最大,GS 平均值为 0.490。与染色体数目为 36 的细胞型间相似程度最高,GS 平均值为 0.530,该值甚至高于峨眉蕺菜种内 GS 平均值 0.521。不同细胞型蕺菜类间 GS 值以染色体数目为 36 和 126 的细胞型间最小(GS = 0.493),即此两类间遗传差异最大。其次是染色体数目 36 和 72 的细胞型类间 GS 平均值 0.524,染色体数目为 82 和 126 的细胞型间的遗传差异最小,其 GS 平均值为 0.633。进一步分析蕺菜 B 至 L 细胞型的类间 GS 值还可发现,其类间 GS 值几乎均是染色体数目为 36 的细胞型间最小,且 B 至 L 细胞型类内遗传相似系数多数小于其类间遗传相似系数,说明这几类细胞型间彼此遗传差异相对较小,而与染色体数目为 36 的细胞型间遗传差异相对较大。

## 2.3 野生类群和栽培类群的遗传相似系数

90 份蕺菜材料中,有 18 份为人工栽培,72 份为野生,其栽培类群类内 GS 平均值为 0.556,变化范围为 0.430 ~ 0.776,变异系数 11.45%;野生类群类内 GS 平均值为 0.577,变化范围为 0.406 ~ 0.848,变异系数 11.17%,栽培与野生类群类间 GS 平均值为 0.566。说明鱼腥草栽培类群间遗传多样性比野生类群间相对更丰富,但二者间的差异还不足以从分子水平上严格区分为两大类。

## 3 RAPD 标记揭示的鱼腥草种质资源间的遗传关系

根据 RAPD 遗传相似系数按 UPGMA 法进行聚类,结果发现,利用 RAPD 标记可将 92 份材料全部区分开。以所有材料间的平均遗传相似系数 0.572 为阈值,可将其划分为 14 类。其中,峨眉蕺菜 W01-86 和 W01-1 分别单独聚为一类。蕺菜中,也有 6 份材料,即 W01-11, W01-22, W01-55, W01-81, W01-94 和 W01-100 分别单独聚为一类,而 W01-4, W01-5, W01-9, W01-16, W01-19, W01-24, W01-25, W01-29, W01-48 和 W01-50 共 10 份材料聚为一类,该类中除 W01-16 和 W01-29 染色体数目分别为 80 和 81 外,其余染色体数目均为 36,主要来自于四川乐山、宜宾和内江地区。W01-39 和 W01-43 聚为一类,其染色体数均为 54,分别来自重庆彭水和四川马尔康。而绝大多数材料,包括 W01-2, W01-3, W01-10, W01-12 等在内的 62 份材料聚为一类,该类各材料染色体数目变化较大,包括作者通过细胞学检测到的各种蕺菜居群染

色体数目,且来源地分布较广,涉及绝大多数材料采集地。其中,来自四川雅安、剑阁、广元、资阳等相同地点的材料大多分别比较近地聚在一起。来自四川芦山的 W01-95 和 W01-97 单独聚为一类,其染色体数目分别为 54 和 90。来自贵州松桃的 W01-33,重庆秀山的 W01-34,重庆酉阳的 W01-37,江苏南京的 W01-114 以及四川都江堰的 W01-46 聚为另一类,其染色体数目在 81 至 90 间变化。此外, W01-6, W01-7 和 W01-18 聚在一起,其染色体数目为 81, 81 和 83, 分别来自四川犍为、沐川和高县。

## 4 RAPD 聚类结果与地理分布的关系

对根据 RAPD 遗传相似系数聚成的 14 类,分析其地理分布情况,可见,由 62 份材料聚成的大类主要分布在四川盆地中部的平原及四周丘陵和低山地区,越靠近盆周山区和边缘地带,类型变化越丰富。重庆黔江、秀山及其相邻的贵州松桃等地则明显地为另一类群。说明鱼腥草根据 RAPD 标记划分的类群同地理分布有一定关系。

## 讨 论

利用随机引物对不同物种基因组 DNA 进行扩增,其多态性表现不同。如石斛多态性为 91.2%<sup>[7]</sup>,玉米自交系间为 59%<sup>[12]</sup>,向日葵种质资源间为 87.2%<sup>[13]</sup>,野山参为 67.6%<sup>[4]</sup>。本研究结果表明鱼腥草种质资源间多态性为 93.5%。推测鱼腥草种质资源基因组 DNA 多态性非常高的原因,主要是由于鱼腥草长期生长在不同地理区域,较少异地引种,在逐渐适应各自所处环境过程中,遗传物质发生了变化,从而形成各具不同遗传特点的类型。

峨眉蕺菜与蕺菜种间 GS 平均值为 0.517,小于峨眉蕺菜和蕺菜各自种内 GS 平均值。也就是说,其种间遗传距离大于种内遗传距离,说明峨眉蕺菜与蕺菜在分子水平上确实存在较大遗传差异。根据细胞学观察,峨眉蕺菜染色体数目为 36,它与蕺菜中染色体数目为 36 的细胞型间 GS 平均值(0.530)最大,故推测二者间亲缘关系最近,也许其本身就是由染色体数目为 36 的蕺菜直接进化而来,或者二者本来就属于一个类群。实质上,这二者间 GS 值为 0.531,大于峨眉蕺菜种内 GS 值 0.521。同时,蕺菜中,染色体数目为 54, 72, 80, 81, 82, 83, 84, 86, 88, 90 和 126 的细胞型几乎均是彼此间遗传差异相对较小,而与染色体数为 36 的细胞型间遗传差异相对较大。祝正银等在区分蕺菜和峨眉蕺菜时,依据的主要鉴别特

征是茎叶颜色、花序长度、粗度、花的数目以及总苞片数等<sup>[3]</sup>,但据作者观察,蕺菜的这几个特征有分化,但性状间无明显间断。如作者在四川宜宾采集资源时,曾发现当地称青侧耳根的材料,其染色体数目也为 36,茎叶偏绿至绿白色,仅略带淡红色。据此认为,峨眉蕺菜在新种划分及其标准上尚值得进一步探讨与商榷。

除染色体数目为 36 的蕺菜细胞型外,其余蕺菜细胞型类内 GS 值表现出染色体数目较少时平均 GS 值相对较小,染色体数目达 80 以上时平均 GS 值相对较大。这可能与随着染色体数目增多、倍性增大,某些位点的基因突变或缺失,部分染色体片段丢失,甚至增加或丢失一至几条染色体均可被其它同源或部分同源染色体所补偿有关。本试验的蕺菜材料中,染色体数目为 36 的蕺菜细胞型内 GS 值较大,可能与供试此类蕺菜材料来源地相对集中有关,故尚需扩大资源收集范围以作进一步分析。

已有研究表明,野生资源往往比栽培种间遗传多样性更大<sup>[4]</sup>。但本试验结果表明,蕺菜栽培类群间遗传多样性相对高于野生类群间。这可能主要因其以种根繁殖为主,人工栽培用种多是近年来通过就地野生驯化而来,很少异地远距离引种等,在驯化过程中人们往往开展了有意识的选择,从大量野生类群中筛选出符合自己需要的生产用种,从而形成了许多各具不同特异性状的栽培类群,一定程度上扩大了栽培类群间的遗传多样性。

聚类分析表明,鱼腥草根据 RAPD 标记划分的类型同地理分布有一定关系,地理位置较近的材料多数能聚在一起。如来自四川雅安的材料,不论其染色体数目多少,绝大多数聚在一起,来自四川芦山、名山、内江、宜宾、资阳、广元和剑阁的材料多数分别聚在一起,来自重庆酉阳、秀山及其相邻的贵州松桃的材料也聚在一起。而盆周山区遗传多样性相对较丰富,可能与盆周山区地形复杂,气候变化较大等有关。由此可以看出,药材道地性与环境有一定关系。遗传相似系数和聚类分析结果还表明,利用 RAPD 技术可将鱼腥草所有供试材料全部区分开,这为 RAPD 技术用于构建鱼腥草 DNA 指纹图谱奠定了良好基础。同时也进一步阐明了鱼腥草种质资源在分子水平上确实存在遗传差异。作者曾将分别来自四川芦山(W01-95)和眉山的栽培蕺菜以及分别来自四川邛崃(W01-100)、雅安望渔(W01-94)和雅安严桥

(W01-113)的野生蕺菜在四川雅安严桥镇同田较大面积种植,结果发现其植株形态、产量及抗病性等均有较大差异,甲基正壬酮含量差异也较大(另文报道),且原产地含量较高者,此处种植后,含量仍属较高者。据此认为,鱼腥草药材的道地性归根到底可能更大程度上由其遗传因素所决定,这为进一步开展鱼腥草新品种选育奠定了理论基础。

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## RAPD ANALYSIS ON THE GERMPLASM RESOURCES OF HERBA HOUTTYNIAE

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**ABSTRACT:** **AIM** To discuss the genetic diversity of the germplasm resources of *Herba Houttyniae* on molecular level. **METHODS** The genetic diversity of 92 *Houttynia* Thunb. accessions were tested by RAPD markers. **RESULTS** Of 34 10-mer arbitrary primers, 32 (94.1%) were found to amplify polymorphic products. A total of 200 products were amplified of which, 93.5% products were found to be polymorphic. 5.8 polymorphic bands were amplified by each polymorphic primers on the average. The average genetic similarity (GS) within *Houttynia cordata* Thunb. and *Houttynia eneiensis* Z.Y. Zhu et S.L. Zhang accessions were 0.521 and 0.572, respectively. The GS between them was 0.517. The degree of similarity of *Houttynia eneiensis* Z.Y. Zhu et S.L. Zhang accessions and *Houttynia cordata* Thunb. cytotype which had the chromosome number of 36 was the highest, with an average of 0.530. The genetic diversity of cultivated groups was higher than that of wild ones. The results of cluster analysis by using UPGMA method showed that all the tested accessions can be differentiated by RAPD markers, and classified as 14 groups. Of which, 62 accessions was classified as one group. The results also showed that the genetic diversity in *Herba Houttyniae* based on RAPD GS was correlated with geographic distribution. **CONCLUSION** (1) There actually existed much genetic diversity on molecular level among the germplasm resources of *Herba Houttyniae*. (2) RAPD markers could be effective tools to construct DNA fingerprintings of *Herba Houttyniae*. (3) The genuineness of *Herba Houttyniae* was related with the environments. However, it was affected by genetic factors in more degree.

**KEY WORDS:** *Herba Houttyniae*; *Houttynia cordata*; *Houttynia eneiensis*; RAPD; genetic diversity; DNA fingerprintings; genuineness