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基于SSR标记分析小豆及其近缘植物的遗传关系

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摘要: 本研究利用87对SSR引物分析了80份栽培小豆(*Vigna angularis*)、22份野生小豆(*V. angularis* var. *nipponensis*)以及10份豇豆属(共7个种)近缘植物, 旨在比较豇豆属不同种的遗传多样性, 并分析种间的遗传关系。结果显示87对SSR引物在112份小豆及其近缘植物资源中共检测到667个等位变异, 其中有75个、71个和82个SSR位点分别在栽培小豆、野生小豆和近缘植物中表现为多态。随机抽样分析发现, 平均每SSR位点检测到的等位变异数目为近缘植物＞野生小豆＞栽培小豆, 与多态信息含量(*PIC*)值一致, 说明近缘植物及野生小豆中蕴含着丰富的遗传变异, 是栽培小豆育种的重要基因来源。聚类分析显示, 栽培小豆、野生小豆和近缘植物间的遗传分化比较明显, 分别聚为三大类, 其中栽培小豆的遗传背景与其生态环境相对应; 近缘植物又可以分为三个亚类, 亚类间的遗传距离与其亲缘关系相对应。本研究结果也说明利用SSR标记辅助豇豆属的种间分类是可行的。

关键词: 豇豆属植物, SSR, 遗传多样性, 遗传关系

Genetic diversity in adzuki bean and its relatives based on SSR markers

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Abstract: In order to enhance the use of genetic resources of *Vigna* in breeding of adzuki bean, genetic diversity and relationship were analyzed based on 87 SSR markers. The results showed 667 alleles were detected at these SSR loci among 112 accessions of *Vigna* plants, including 80 cultivated, 22 wild adzuki bean and 10 relative plants. And 75, 71 and 82 were polymorphic in cultivated, wild adzuki bean and relative plants, respectively. Random sampling analysis suggested that the relative plants ranked the first for the average number of alleles per SSR locus, and wild adzuki bean ranked the second, this was agreeable with polymorphism information content (*PIC*). Cluster analysis divided the cultivated, wild adzuki bean and relative plants into three distinct groups. The genetic background of cultivated adzuki bean basically agreed with their origins. The relative plants could also be divided into three sub-groups, and *V. umbellata* had different genetic background with other wild species. The present study suggested that wild *Vigna* had higher genetic variations than cultivated adzuki bean, and could be used to broadening the gene pool of this crop.

Key words: adzuki bean, *Vigna*, genetic diversity, SSR

小豆(*Vigna angularis*)是豇豆属的一个栽培种, 也是我国传统的出口创汇商品(郑卓杰, 1995)。近年来, 新品种选育的步伐缓慢, 难以满足市场对小豆产品多元化的需求。2009年初, 国家食用豆产业技术体系在对全国小豆各行业技术需求的调研中发现, 对抗病虫、抗逆、高产、优质品种的需求排在第一位。目前我国已收集保存小豆种质资源5,000余份, 其中3,000余份已完成编目(郑卓杰, 1987, 1990; 胡家蓬等, 1996)。这些资源中, 国内材料占98%以上, 国外材料主要来源于澳大利亚、泰国和日本等国。我国小豆种质资源不仅有丰富的表型变异(田静和赵春霞, 2001; 魏淑红等, 2004; 王述民等, 2002a), DNA分子标记水平的遗传变异也较高(Yee *et al*., 1999; 王述民等, 2002b; 粟生群等, 2005;

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叶剑等, 2008; 王丽侠等, 2009)。然而这些栽培小豆种质资源中抗病虫性、抗逆性、优质等有益基因资源比较缺乏(刘长友等, 2009), 使现阶段小豆育种难以取得突破性进展。因此寻找新的抗性、优质基因对丰富小豆基因库非常重要(喻少帆等, 1997; 魏淑红, 1998; 陈学珍等, 2001)。近年来, 大豆(齐宁等, 2006; 杨光宇等, 2007)、水稻(赵卫东等, 2007)、小麦(任志龙等, 2007)等的近缘野生种的抗病、高产等优异基因在育种中得到利用, 野生绿豆(*Vigna radiata* var. *sublobata*)中抗豆象基因也进行了育种利用(Tomooka *et al*., 1992; Lambrides & Imrie, 2000; Lin *et al*., 2005)。因此, 深入发掘野生小豆及豇豆属野生近缘植物资源中的优异基因, 可能对栽培小豆遗传育种工作取得突破性进展起到关键作用。

然而, 我国野生小豆及近缘植物的收集工作相对滞后。从2005年开始在全国范围内开展了豇豆属近缘野生植物资源的考察。考察推测, 这些野生资源中可能存在多荚、耐旱、抗病等优良基因资源。由于豇豆属野生近缘植物间表型性状差异较小, 难以准确归类, 导致育种利用存在困难。利用DNA分子标记进行野生近缘种分类已有进展(杨萍等, 2004; 田松杰等, 2004; 蔡青等, 2005; 张冰冰等, 2008), 尤其是SSR标记, 具有共显性、操作简单、费用低等优点, 在遗传多样性研究中的应用非常广泛。本研究利用公开发表的小豆SSR标记(Wang *et al*., 2004; Han *et al*., 2005), 分别对栽培小豆、野生小豆和近缘植物进行了遗传多样性比较分析及遗传关系的探讨, 以期为我国小豆种质资源的评价鉴定及其野生近缘种中优异基因的利用提供参考。

1 材料与方法

1.1 实验材料

112份材料分为栽培小豆、野生小豆和近缘植物3类。其中栽培小豆80份来自小豆核心种质库, 分别从国内14个省份随机选取77份(包括吉林9份、河北9份、北京9份、天津6份、山西4份、内蒙5份、黑龙江7份、江苏6份、辽宁6份、湖北5份、河南2份、安徽4份、云南3份、陕西2份), 从国外引进资源中随机选取3份。野生小豆22份和近缘植物10份(其中豇豆3份及野生种7份)均由日本引进(表1)。

表1 小豆及其近缘植物的类别及受检种质数

Table 1 Number of accessions detected for different species in *Vigna*

种属 Species	数量 Amount
Vigna angularis	80
V. angularis var. *nipponensis*	22
V. umbellata	3
V. hirtella	1
V. tenuicaulis	1
V. riukivernsis	2
V. minima	1
V. trinervia	1
V. nakashimae	1
合计 Total	112

1.2 DNA提取及SSR分析

选取每份小豆种质2–3粒健壮种子播种于营养钵内, 采集第一个三出复叶刚展开的幼嫩叶片, 用CTAB法提取基因组总DNA(Doyle & Doyle, 1987)。经紫外分光光度计测定浓度后, 加ddH_2O稀释至10 ng/μL的工作浓度, 备用。

选择扩增带谱比较清晰的小豆SSR引物87对(Wang *et al*., 2004; Han *et al*., 2005)。PCR扩增反应在Perkin-Elmer GeneAmp PCR System 9600热循环仪上进行。反应体积为20 μL, 包含1×PCR缓冲液, 2 mM $MgCl_2$, 0.1 mM dNTP, 0.4 μM的引物, 20 ng DNA和1U *Taq* DNA聚合酶。反应程序为: 95℃预变性5 min; 94℃变性30 s, 退火30 s, 72℃延伸30 s, 循环35次; 72℃延伸5 min。用6%的变性聚丙烯酰胺凝胶电泳分离扩增产物, 银染法染色(Bassam *et al*., 1991; Vantoai *et al*., 1996)。

1.3 数据处理

按照各SSR位点PCR扩增片段即等位变异迁移率的不同分别读取数据, 并依据分析软件的要求相应转换数据格式。用非加权成组配对算术平均法(unweighted pair-group method with arithmetic mean, UPGMA)计算种质间Nei-Li遗传相似性系数, 用NTSYS-pc2.10作主成分分析(principal coordinates analysis, PCO)及树状聚类图(Rohlf, 1992); 利用PopGen32软件包计算多态信息含量(polymorphism information content, *PIC*)(Yeh & Boyle, 1997); 用浙江大学徐海明开发的ALLELENUM程序(未发表)作随机抽样分析以削弱样本大小对遗传多样性评价的影响。其他统计分析和绘图均在Excel中完成。

2　结果

2.1　遗传多样性

87对SSR引物在112份小豆及其近缘植物中共检测到667个等位变异, 每对引物检测到等位变异的数目从2个到22个, 平均为7.58个。其中等位变异最丰富(22个)的引物为X25(LG1)和X167(LG9); 仅检测到2个等位变异的引物共4对, 占4.6%。等位变异频率从0.89%到95.54%不等, 平均为11.96%, 频率高于50.0%的等位变异为49个, 占7.35%, 即有49对SSR引物检测出优势等位变异; 分布频率低于5%的等位变异占60.42%。多态信息含量的变异从0.036到0.942, 平均为0.504。其中多态信息含量最高的引物是X25, 最低的是X74, 分别位于小豆的第1和第4连锁群。多态信息含量高于0.5的SSR为53个, 占60.9%。

2.2　不同群体遗传变异的分布与比较

在所检测的87个SSR位点中, 有75个、71个和82个分别在栽培小豆、野生小豆和其他近缘植物群体内具有多态性。栽培小豆、野生小豆、近缘植物群体每SSR位点的平均等位变异数分别为4.9个、3.7个和5.1个, 平均多态信息含量分别为0.48、0.54和0.62。随机抽样分析发现, 当样本数为22时, 栽培小豆的平均等位变异数为2.7个, 小于22份野生小豆的平均等位变异数(3.7); 当样本数为10时, 栽培小豆和野生小豆的等位变异数分别为1.9个和3.0个, 均低于10份近缘植物(5.1个)。

在667个等位变异中, 栽培小豆、野生小豆和近缘植物的特有等位变异分别有94、45和214个; 栽培小豆和野生小豆的共有等位变异最多, 近缘群体和野生小豆的共有等位变异最少(图1)。

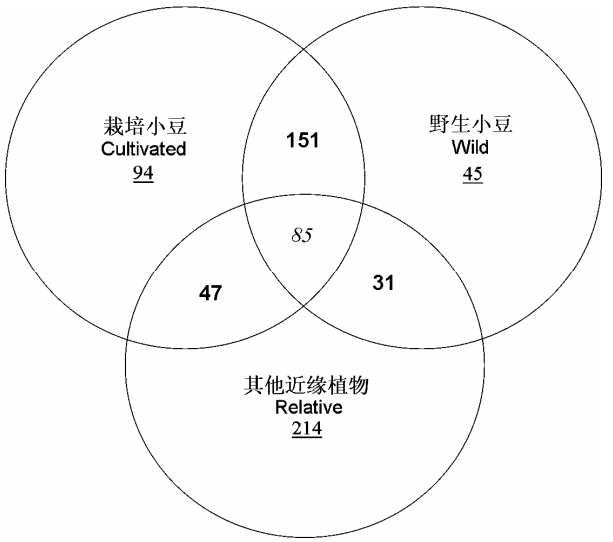

图1　667个等位变异在不同小豆或近缘植物群体间的分布。下划线者为群体特有等位变异, 黑体为两两群体间特有等位变异, 斜体为3个群体间共有等位变异。

Fig. 1　Distribution of 667 alleles among cultivated, wild adzuki bean and other relatives. The underlined number is the specific alleles within each population, the bold numbers are the common alleles between populations, and the italic number is the specific alleles among the three populations.

2.3　遗传分化与遗传关系分析

基于UPGMA的聚类分析显示, 栽培小豆(I)、野生小豆(II)、近缘植物(III)能够完全分开(图2)。80份栽培小豆大致又可以分为4个组, 其中第一组群(i)的27份种质以华北地区最多, 占81.5%; 第二组群(ii)的27份种质以东北地区最多, 占74.1%; 第三组群(iii)的10份种质中有8份来自安徽和江苏; 第四组群(iv)中的16份种质来源比较广泛, 包括湖北5份、北京3份、吉林2份、云南2份、国外2份、山西和辽宁各1份。

近缘植物群体可以分为三亚类, 其中3份栽培饭豆(*Vigna umbellata*)聚成第一亚类, 且与野生小豆的遗传距离相对较近; *V. hirtella*、*V. tenuicaulis*组成第二亚类; 而*V. riukiuensis*、*V. nakashimae*、*V. minima*、*V. trinervia*为第三亚类。聚类分析结果与基于主成分分析的二维坐标图相吻合(图3)。

3　讨论

3.1　不同群体的遗传多样性

研究表明, 我国喜马拉雅山脉及其周边的野生小豆群体比栽培小豆具有更高的遗传变异(Zong *et al*., 2003; Xu *et al*., 2008)。87对SSR引物在80份栽培小豆、22份野生小豆和10份近缘植物群体中检测到的平均等位变异数分别为4.9、3.7和5.1个。由于样本量相差悬殊, 这一结果缺乏可比性, 因为在样本量较小的情况下, 检测到的等位变异数往往与样本量成正比(王丽侠等, 2004)。因此为减少样本量对遗传多样性评价结果的准确性的影响, 本研究采用了随机抽样的分析方法, 结果发现当样本量相同时, 平均等位变异数依次为近缘植物＞野生小豆＞栽培小豆, 且差异极显著, 平均多态信息含量也为近缘植物＞野生小豆＞栽培小豆。可见, 野生小豆和

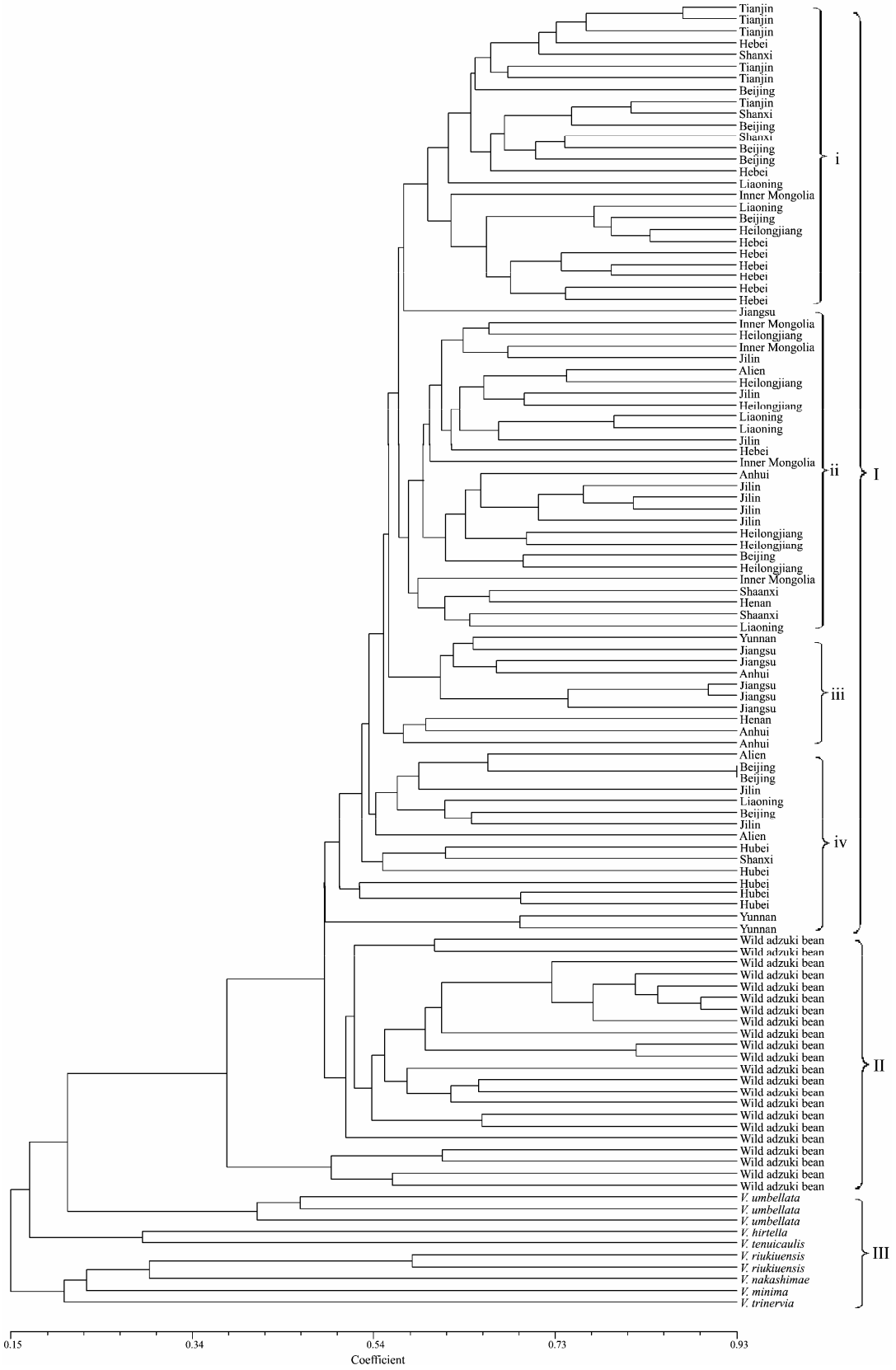

图2 基于小豆及其近缘植物间遗传相似性系数绘制的树状聚类图

Fig. 2 Dendrogram showing genetic relationship among adzuki bean and its relatives based on coefficient of similarity

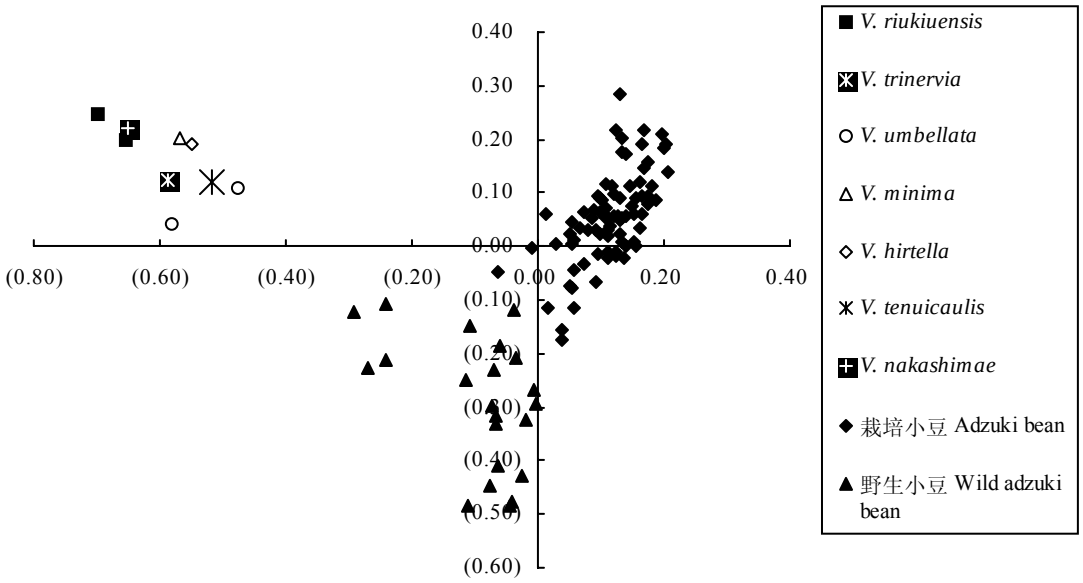

图3　基于主成分分析绘制的小豆及其近缘植物关系的二维坐标图
Fig. 3　Two-dimentional representaion of genetic relationship among adzuki bean and its relatives

小豆其他近缘植物具有更高的遗传变异, 可作为栽培小豆基因资源的有益补充。

3.2　种内及种间遗传背景及遗传关系分析

种质资源的遗传背景往往与其生境相关(Wang *et al*., 2006), 而我国至今还没有公认的小豆生态区划标准(胡家蓬, 1984; 金文林, 1995)。王述民等(2002a)利用RAPD标记对我国小豆种质资源的聚类分析表明, 小豆组群的划分与地理来源无明显关系, 推测是由所用RAPD标记数量太少, 揭示的信息量有限所致。因为在随后的AFLP分析中发现小豆种质资源的遗传背景与其地理来源有一定的相关性(王述民和张赤红, 2002)。王丽侠等(2009)分析发现我国小豆种质资源的遗传背景与其地理来源有较大的一致性, 并认为我国小豆生态区划可分为东北、华北、华东、华中和西南5个区。本研究对栽培小豆的聚类分析基本支持这一观点。关于国内不同来源小豆种质资源的遗传多样性, 本文未作相关的比较, 主要原因在于国内小豆种质资源的取样量过少。但是本研究对国内不同来源小豆遗传背景的评价以及积累的分子标记数据, 将对资源的再收集、鉴定及新基因发掘、种质创新等研究提供信息, 也有利于不同地区制定相应的小豆育种策略, 尤其是亲本选择与组配以及后代的辅助鉴定等。

栽培小豆与豇豆属的其他栽培种或者野生近缘植物大多存在生殖隔离, 然而近期的研究表明利用桥梁作物可能克服生殖隔离, 实现抗性基因在栽培种间的转移(Somta *et al*., 2006)。本研究的分析材料均为豇豆属亚洲亚属中*Angularis*类型, 由聚类结果可以看出, *V. trinervia*与其他材料的遗传关系最远, 这进一步验证了其分类学地位, 即介于*Angularis*和*Ceratotropis*类型之间(Tateishi, 1985), 可能作为两者间重要的桥梁亲本。另外, 栽培小豆与野生小豆间的遗传距离远远小于与其他近缘植物间, 且二者间不存在生殖隔离, 因此, 加强野生小豆优异基因资源的利用极其重要。

3.3　野生近缘植物的育种利用

本研究中小豆近缘植物中有214个等位变异是栽培小豆和野生小豆所不具备的, 这些等位变异是否与一定的基因功能有关, 还需要进一步验证。相关研究已表明豇豆属作物近缘植物中蕴含着丰富的优良抗性基因资源, 比如*V. nakashimae*具有较强的茎腐病抗性(Kaga *et al*., 2000), *V. trinervia*具有豆象抗性(Credland, 1986)。本课题组近期考察发现, 我国境内分布有丰富的野生小豆及野生近缘植物资源。为加强这些豇豆属野生资源的评价分析, 本课题组在形态学观察的基础上, 已经着手利用染色

体核型分析、DNA分子标记等方法对收集到的近缘植物进行明确归类, 并开展了抗豆象鉴定和种间杂交等研究, 目前已经有所进展。

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