Identification of closely related mango cultivars by ISSR

HE Xin-Hua[1,2], LI Yang-Rui[1]*, GUO Yong-Ze[2],
OU Shi-Jin[2], LI Rong-Bai[1]

(1. *Laboratory of Guangxi Crop Genetic Improvement and Biotechnology, Guangxi Academy of Agricultural Sciences*, Nanning 530007, China; 2. *College of Agriculture, Guangxi University*, Nanning 530004, China)

Abstract: Seven carabao mango cultivars or lines and Liuzhou lusong mango(*Mangifera indica* Linn) were examined with ISSR primers. Of the 30 primers screened, 6 primers gave reproducible, polymorphic DNA amplification patterns, and were selected to construct a DNA fingerprinting map to distinguished carabao mango cultivars or lines. According to the banding patterns, all cultivars tested in this study were distinguished from each other by every one of 6 selected primers and showed ample genetic diversity, indicating that ISSR-PCR was an effective method for cultivar identification of mango cultivars and lines. Based on UPGMA analysis of 69 selected bands, the carabao showed the lowest similarity to all other cultivars while Gaozhou carabao, Zhanjiang carabao, Tianyang xiangmang, Jinqianmang, Liuzhou lusong, Yueximang No. 1 and Panxi red carabao could be clustered into one group.

Key words: mango (*Mangifera indica*); ISSR; cultivar identification

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Molecular markers provide an attractive and more reliable alternative to morphological markers. In mango (*Mangifera indica* Linn), phenotypic markers have been the most common method for describing cultivars. Although this approach is useful to distinguish distantly related cultivars, its reliable application has proven more difficulties when it comes to differentiating closely related lines or off types of a particular cultivar. Therefore, a more refined technique, such as molecular markers that are highly polymorphic, is required.

In the last decade, many molecular methods have been implemented in mango, but random amplified polymorphic DNA(RAPDs)(Bally *et al.*, 1996; Deng *et al.*, 1999; Karihaloo *et al.*, 2003; Lopez-Valenzuela *et al.*, 1997; Ravishankar *et al.*, 2000; Schnell *et al.*, 1992, 1995; Xu *et al.*, 1998) and amplified fragment length polymorphism(AFLPs)(Eiadthong *et al.*, 2000; Fang *et al.*, 1999, 2000, 2001) were more commonly used, and the two methods have also been applied in discrimination of mango cultivars in China(Deng *et al.*, 1999; Fang *et al.*, 1999, 2000, 2001; Xu *et al.*, 1998). Among these molecular methods, inter-simple-sequence-repeats (ISSRs) is a relatively novel technique and has been proven to be a powerful, rapid, simple and inexpensive way to assess genetic diversity(Fang *et al.*, 1997a) or to identify closely related cultivars(Fang *et al.*, 1997b) and to study evolutionary processes in tree species(Wolfe *et al.*, 1998). ISSRs have been applied in identification of mango in Thailand(Eiadthong *et al.*, 1999) and in Australia(Gonzalez *et al.*, 2002), but have not been reported in China yet.

Carabao introduced from Philippines was widely planted in our mango cultivation areas, and many new lines have been selected from carabao for several decades, while there is much confusion and difficulty in i-

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作者简介: 何新华(1966-), 男, 湖南衡阳人, 博士, 教授, 研究方向: 果树生物技术。

* 通讯作者(Author for correspondence)

dentifying the existing carabao cultivars in China. Thus, in the present study, ISSRs were used to discriminate carabao cultivars or lines.

1 Materials and methods

1.1 Plant materials

Gaozhou carabao, Zhanjiang carabao, Tianyang xiangmang(Seedling Variation of Carabao), Jinqianmang and Liuzhou lusong(variation of Philippine mango variety) from the germplasm collections of Guangxi Academy of Agricultural Sciences, and Carabao, Yueximang No. 1 (Seedling Variation of Carabao) and Panxi red carabao (Seedling Variation of Carabao) from the germplasm collections of Subtropical Plant Institute of Guangxi were employed as the plant materials in the present study.

1.2 DNA extraction

DNA was extracted from mango leaves using the Cetyl Trimethyl Ammonium Bromide (CTAB) method described by Doyle and Doyle (1990) with a little modification. Leaf samples of 0.5 g were ground to fine power in liquid nitrogen and added into a 10 mL tube with 4 mL preheated (65 ℃) extraction buffer consisting of 2% CTAB, 1.4 mol/L NaCl, 0.1% (v/v) β-mercaptoethanol, 20 mmol/L EDTA, 100 mmol/L Tris-HCl (pH8.0), and 1%(w/v)PVP-40. The homogenates were incubated at 65 ℃ for 1 h and extracted one time with 4 mL chloroform:isoamyl alcohol (24 : 1) solution. The tubes were mixed for about 5 min, and centrifuged at 12 000 rpm for 10 min. The supernatant was transferred into a new tube containing twice volume of 100% ethanol and 1/10 volume 3 mol/L NaAc, mixed gently, left at -20 ℃ for 1h and spun at 12 000 rpm for 15 min. The supernatant was then discarded and the pellet washed twice with 70% ethanol. The pellet was dried at room temperature, and resuspended into 200 μL 0.1×TE with RNase A. The DNA was precipitated with 400μL 100% ethanol at -20 ℃ for 1 h and centrifuged at 12 000 rpm for 15 min. The pellet was resuspended into 100 μL 0.1×TE. The DNA concentrations were detected by Eppendorf Biophotometer.

1.3 ISSR analysis

ISSRs primers were designed by ourselves or referred to the papers of Eiadthong *et al.* (1999) and Gonzalez *et al.* (2002) and synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. Thirty primers were screened in the present study. Each 20 μL amplification reaction consisted of 10 mmol/L Tris-HCl(pH8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L mixed dNTP, 0.25 μmol/L primer, 1 unit *r*Taq polymerase (Takara Biotechnology, Japan), and approximately 60 ng genomic DNA. Amplification was performed in Thermcycler under the following conditions: 5 min at 94 ℃ for 1 cycle, followed by 1 min at 92 ℃, 1 min at 47 ℃ and 2 min at 72 ℃ for 40 cycles, and 10 min at 72 ℃ for final extension. The amplification products were loaded onto a 2.0% agarose gel in 1×TBE buffer for electrophoresis at 100 V for 1 h, and then visualized by staining the gel with ethidium bromide. The size of each fragment was estimated with reference to GeneRuler™ 100 bp DNA ladder plus (MBI).

1.4 Data analysis

Eighty reproducible bands from selected primers were scored as 1(presence) or 0(absence) for 8 cultivars or lines tested. Then, the unweighted pair-group method using arithmetic averages(UPGMA) cluster analysis was performed using NTSYSpc version 2.1e software(Numerical Taxonomy System version 2.01).

2 Results and analysis

2.1 Screening of primers and diversity analysis of mango genomic DNA

Of the 30 ISSR primers screened, 6 primers(Table 1) were selected in our analyses for their reproducible and polymorphic DNA amplification patterns (Fig. 1). The PCR by 6 ISSR primers yielded a total 80 bands and 69 scorable polymorphic markers (Table 1), accounting for 86.3% of total. Each primer could produce 7 to 16 DNA polymorphic bands, and the size of bands amplified was between 200 bp to 2 800 bp.

According to the banding patterns obtained with 6 selected primers, all carabao cultivars tested in this study could be distinguished from each other, indicating that ISSR-PCR was an effective method for cultivar identification of mango, and the genetic diversity of carabao cultivars or lines was clarified by comparing 69 polymorphic

loci(Table 1).

Table 1 Sequence of reliable ISSR primers and the number of scorable polymorphic bands of each primer

Primers*	Sequence (5′-3′)	Total amplified bands	Number of polymorphic bands	Percentage of polymorphic bands
UBC-840	$(GA)_8YT$	13	10	76.9
UBC-841	$(GA)_8YC$	17	16	94.1
UBC-851	$(GT)_8YG$	15	14	93.3
UBC-857	$(AC)_8YG$	8	7	87.5
UBC-876	$(GATA)_2$ $(GACA)_2$	14	14	100.0
GXU-1	$(ACACACAT)_2$	13	8	61.5
Total		80	69	

Note: * "UBC" was stood for primers designed by Biotechnology Laboratory, University of British Columbia, Canada; "GXU" represented primer designed by ourselves

2.2 Cluster analysis

The dendrogram obtained by the UPGMA clustering method revealed the genetic relationship of 8 mango cultivars tested in this study(Fig. 2). On the dendrogram, the carabao showed the lowest similarity to all other cultivars while Gaozhou carabao, Zhanjiang carabao, Tianyang xiangmang, Jinqianmang, Liuzhou lusong, Yueximang No. 1 and Panxi red carabao could be clustered into one group.

3 Discussion

In the previous papers, it was told that the Gaozhou carabao and Zhanjiang carabao are the same cultivar with different names as carabao introduced from Philippine, and Tianyang xiangmang, Jinqianmang, Yueximang No. 1 and Panxi red carabao are lines or cultivars selected from carabao, so there is much confusion and difficulty in identifying these carabao

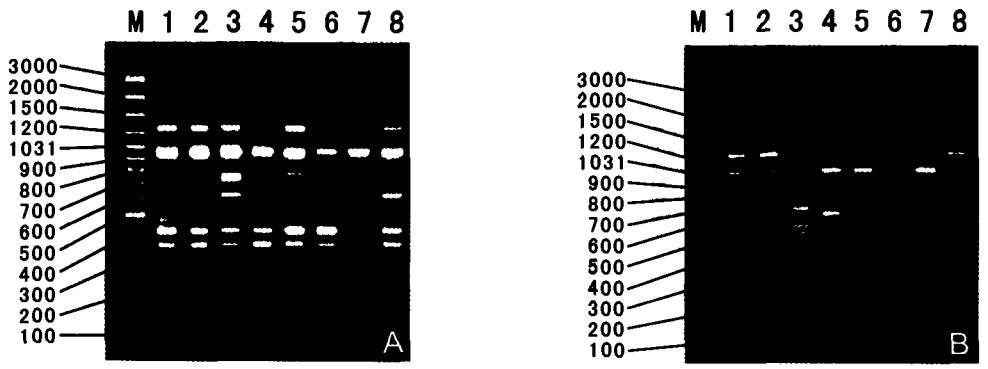

Fig. 1 PCR amplified patterns by UBC-840(Fig. A)and UBC-841(Fig. B)primer in eight carabao cultivars.
Lane M: GeneRuler™100 bp DNA ladder plus; **Lane 1-8**: Represented for cultivars Gaozhou carabao, Zhanjiang carabao, Tianyang xiangmang, Jinqianmang, Liuzhou lusong, Carabao, Panxi red carabao, and Yueximang No. 1 respectively.

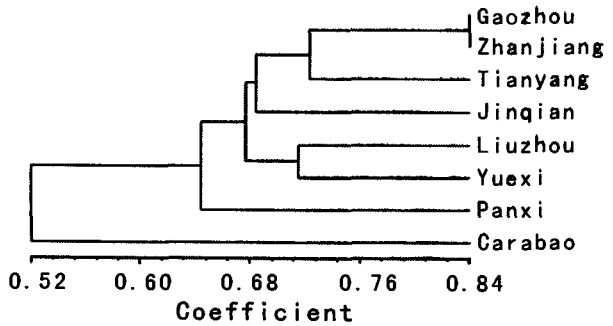

Fig. 2 Dendrogram of phylogenetic relationship among 8 carabao cultivars or lines by ISSR markers based on UPGMA analysis

confusion and difficulty in identifying these carabao cultivars by morphological markers. But they showed distinct DNA amplification patterns and high polymorphism by ISSR with one of the 6 selected primers in the present study, it was very easy to distinguish them. ISSR technique in this study seems to be quite valuable for identification of carabao mango cultivars or lines and evaluation of their genetic diversity.

The result indicated that the carabao showed the lowest similarity to the other cultivars in the study, the reasons may be as follows: (1)the carabao in the study might not be the true carabao originated in Philippines; (2)the carabao in the study could be mutant line of carabao from Philippines because of top-grafting and environmental factors.

The Liuzhou lusong originated in Philippines was a cultivar different from carabao, but it showed high similarity to other carabao cultivars or lines in the present

study, especially the similarity between Liuzhou lusong and Yueximang No. 1 were more than 70%, indicating that it had close relationship with other carabao cultivars. Therefore, Liuzhou lusong could belong to a cultivar or line of carabao type.

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ISSR鉴定亲缘关系非常近的芒果栽培品种

何新华[1,2]，李杨瑞[1]*，郭永泽[2]，欧世金[2]，李荣柏[1]

(1.广西农业科学院 广西作物遗传改良生物技术重点开放实验室，南宁 530007；2.广西大学 农学院，南宁 530004)

摘 要：用ISSR技术鉴定7个吕宋芒品种(系)和柳州吕宋芒。从30个引物中筛选出6个多态性好的ISSR引物建立DNA指纹图谱用于区分吕宋芒品种(系)。分析DNA指纹图谱，发现这6个引物中每个引物都能区分吕宋系列品种(系)，表明ISSR-PCR技术对芒果品种(系)的鉴定非常有效，能区分亲缘关系很近的品种(系)。基于69条多态性条带的聚类分析结果，发现吕宋芒和其它供试的7个品种(系)同源性低，而这7个品种(系)：高州吕宋芒、湛江吕宋芒、田阳香芒、金钱芒、柳州吕宋芒、粤西一号、攀西红吕宋同源性较高，可归为一类。

关键词：芒果；ISSR；品种鉴定