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中国30个陆地棉主栽品种的SSR指纹图谱

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摘　要：利用SSR分子标记技术，对中国不同生态棉区曾经或正在种植的主要来源于岱字棉、斯字棉、福字棉、乌干达棉的30个陆地棉主栽品种进行了DNA指纹分析。从1 803对SSR引物中筛选到重复性好、多态性丰富的20对核心引物。这些引物分属棉花15条染色体，共检测到116个等位基因，平均每对引物5.8个；PIC值范围为0.384～0.900，平均为0.716；MI值范围为1.152～9.000，平均为4.374。30个品种中有4个品种具有各自的特异引物，可将其与其它26个品种区分开，其它26个品种可利用至少2对引物组合进行区分。为更方便、准确地鉴定各品种，构建了30个品种20对核心引物的十进制数字指纹代码。该研究为陆地棉的品种鉴定和纯度检测、新品种权益保护以及标准DNA指纹库构建提供了重要依据。

关键词：陆地棉；核心引物；DNA指纹；数字指纹代码

中图分类号：Q789　　**文献标志码**：A

SSR Core Primers Reveal DNA Fingerprinting of 30 Upland Cotton (*Gossypium hirsutum* L.) Varieties in China

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Abstract: DNA fingerprintings of 30 main Upland cotton (*Gossypium hirsutum* L.) varieties were analyzed using SSR core primers. These experimental materials mainly derived from Deltapine, Stoneville, Foster and Uganda pedigrees, and they have been or are being planted in different ecological cotton-growing areas in China. Twenty pairs of 1 803 primers were confirmed as core primers with stable repeatability and rich polymorphism. These primers belonged to 15 cotton chromosomes, and amplified a total of 116 alleles with an average of 5.8. Polymorphism information content (PIC) values ranged from 0.384 to 0.900 with an average of 0.716, and marker index (MI) values ranged from 1.152 to 9.000 with an average of 4.374. Four of the 30 varieties had their specific primers, by which each variety could be distinguished from the other varieties; each of the other 26 varieties could be distinguished from the other varieties by using at least 2 pairs of primer combinations. In order to identify the 30 varieties more conveniently and accurately, their decimal digital fingerprinting codes were also constructed based on their electrophoresis patterns of the 20 core primers. This study provides important references for identification and purity detection of cotton variety, protection of intellectual property rights and standard database construction of DNA fingerprinting of Upland cotton.

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Key words：Upland cotton (*Gossypium hirsutum* L.)；core primers；DNA fingerprinting；digital fingerprinting code

Cotton is an important economic fiber crop, and cotton production has a significance role in global economy[1]. Cotton is a typical plant which is often cross-pollinated with a natural outcrossing rate of 5% to 20%[2]. At present, many cultivated cotton varieties do not maintain satisfactory genetic purity, which greatly affects the extension and further application of released varieties. Therefore, it is necessary to adopt an efficient and accurate detection method for variety purity detection. The traditional identification methods such as seed morphology, seedling and field plot phenotyping have played an important role in variety purity detection. However, with the centralized utilization of current breeding parents, variety identification became more and more difficult. Traditional methods are no longer compatible with the development of variety analysis due to their time-consuming and high-cost characteristics. In the 1970s, some biochemical techniques such as protein isoelectric focusing electrophoresis (IFE), SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and isozyme electrophoresis were successfully applied in variety identification and purity detection of some crops. However, because the proteins and isozymes are gene expression products, the related identification technologies are often affected by environmental conditions and plant developmental stages. Therefore, these methods have rarely been applied at present.

In the 1980s, the rise of various molecular marker techniques based on DNA variation led to the rapid development of DNA fingerprinting. The polymorphism with large quantity, high accuracy, and high individual specificity and environmental stability, revealed by this electropherogram, can directly respond to differences among different genomic DNA. Therefore, it is called "DNA fingerprinting". Using the molecular marker technology to construct DNA fingerprinting has become a powerful tool in variety identification[3]. The studies have been conducted in many important crops such as tomato[4], rape[5], rice[6], wheat[7], maize[8], cucumber[9] and jute[10]. Cotton DNA fingerprinting has made great progress in recent years[11-14]. Among those molecular markers, Simple Sequence Repeat (SSR) has been widely used in studies of cotton genetic diversity, DNA fingerprinting and marker-assisted selection (MAS) because of its advantages such as high polymorphism, stability and repeatability[15].

In this study, DNA fingerprinting of 30 main cultivated Upland cotton varieties in China was analyzed using 20 SSR core primers with stable repeatability and excellent polymorphism. Simultaneously, the decimal digital fingerprinting codes of these materials were also constructed. Our study will provide important references for variety purity detection, intellectual property rights protection and constructing standard DNA fingerprinting database of Upland cotton.

1　Materials and methods

1.1　Plant materials

Thirty main Upland cotton (*Gossypium hirsutum* L.) varieties used in this study were mainly derived from Deltapine, Stoneville, Foster and Uganda pedigrees, and they have been or are being planted in different ecological cotton-growing areas of Yangtse River Valley, Yellow River Valley, and Northwest Inland in China. These varieties were all inherited stably after many generations of self-pollination. The list of all varieties along with their detail pedigrees and released year was provided in Table 1.

1.2　SSR basic primer selection

A total of 1 803 SSR primers were used as basic primers to screen the polymorphism among germplasms. These primers were mainly selected from the published cotton interspecific and intraspecific genetic linkage maps, as well as the reported molecular markers linked with quantitative

Table 1 List of 30 Upland cotton varieties along with their pedigrees and released years

Code	Variety	Pedigree	Released year
1	CIR12	Uganda 4×Xingtai 6871	1989
2	CIR41	CCRI23 with *Bt*+*CpTI* bivalent insect-resistant genes	2002
3	CIR45	961027 with *Bt*+*CpTI* bivalent insect-resistant genes selected from Jin 95-1	2003
4	CIR50	Zhong 394 with *Bt*+*CpTI* bivalent insect-resistant genes	2007
5	CIR58	SGKzhong 27×92-047	2006
6	CIR64	SGKzhong 27×Short-season cotton zhong394	2007
7	Liaomian 19	Liao 205×SGK321	2003
8	Lumianyan 19	Lu 458 line×Simian 3 selection line	2005
9	Lumianyan 21	Shiyuan 321 selection line×Simian 3 selection line R55	2005
10	Lumianyan 22	CCRI19×A line from GK-12 initial lines	2005
11	Lumianyan 28	(Lumian 14×Shiyuan 321) F_1×mixed pollen	2006
12	Lumianyan 29	Lu 735×168 (selected from GK12)	2006
13	Jinmian 29	82-87×84S-14	2000
14	Jinmian 36	Jinmian 19×Yun 148	2003
15	Jinmian 45	Jinmian 13 selection line 94h-14×k338	2006
16	Simian 3	Yankang 76-75×Si791	1993
17	Simian 4	Simian 3×Nantong 84-239	2000
18	Yumian 15	Short-season cotton shang 85-5 selection line	2000
19	Shan 2365	Naturally selected line from Shan 4080	2005
20	Jimian 958	(Jimian 10×538) F_1×Jimian 22	2006
21	Hanmian 802	(85-4.336×CCRI12) F_1×(85-0.044×Jimian 11)F_1	2006
22	Hanmian 885	Handan 284×Handan 109	2006
23	Xuzhou 142	Xuzhou 58 selection line	1973
24	Xuzhou 514	CCRI7×Xuzhou 142	1990
25	Xinqiu 1	Zhong 9418×GK12	2006
26	Baimian 1	Bainong 8602×Xin 3313	2009
27	SGK958	Jinke 970012×Jinke 19	2009
28	Guoxinmian 3	CCRI17 with *Bt*+*CpTI* bivalent insect-resistant genes	2006
29	Kemian 4	2076×679	2005
30	Zhongzhimian 2	Zhongzhi 372 with *Bt* gene	2006

trait loci (QTL) for important agronomic and economic characters in cotton[16-23]. The primer sequences were obtained from Cotton Marker Database (http://www.cottonmarker.org).

1.3 SSR-PCR analysis

All sample materials were planted in cotton breeding station of Henan Institute of Science and Technology in 2010, Xinxiang, Henan, China. Each material was laid out in one row with 13～15 plants (0.8 m wide, 5 m long). DNA from single plant of each material was extracted using the cetyltrimethylammonium bromide (CTAB) method described by Paterson *et al*[24]. The SSR reaction was in a total of 10 μL, containing 10×PCR buffer 1.0 μL, Mg^{2+} (25 mmol/L) 1.0 μL, dNTP (10 mmol/L) 0.2 μL, *Taq* polymerase (5 U) 0.1 μL (Beijing DingGuo Changsheng Biotechnology Co. Ltd), primer F and primer R (5 μmol/L) each for 1.0 μL, template DNA (26 ng·μL^{-1}) 1.0 μL, and ddH_2O 4.7 μL. The protocol for polymerase chain reaction (PCR) amplification and silver staining was referred to Zhang *et al*[25].

1.4 SSR core primers, PIC value and MI value

Core primers are a set of primers with good comprehensive characteristics of polymorphism, stability and repeatability[26]. After confirming the pedigree source, five varieties with a remote relationship, Jinmian 36 (Deltapine pedigree), Xuzhou 142 (Stoneville pedigree), Simian 3 (Foster pedigree), CIR12 (Uganda pedigree) and Lumian 21 (Uganda pedigree) were firstly selected to screen all of 1 803 SSR primers. The polymorphic primers

obtained were then used for further screening of all materials. Ultimately, the SSR primers with clear and stable bands, and having 3 or more alleles, were selected as core primers. The bands of PAGE were converted into digital fingerprinting patterns as 1 for presence and 0 for absence in the same migration position. The polymorphism information content (PIC) value was computed by the following formula:

$$PIC_i = 1 - \sum P_{ij}^2$$

where, P_{ij} represents the frequency of the jth allele of marker i in population[27]. Referring to Smith *et al*[28], marker index (MI) value was calculated as:

$$MI = \text{Allele} \times PIC$$

where, allele is the number of primer allele. The core primers were confirmed through a comprehensive consideration of the number of allele, *PIC* value and *MI* value of the primer.

1.5 DNA fingerprinting analysis

According to the electrophoresis pattern of core primers obtained as mentioned above, the specific primers or primer combinations were identified, by which one material could be distinguished from any other variety. In a more simple and convenient way, the digital fingerprinting codes of all materials were also constructed by converting "0/1" binary data to decimal data.

2 Results and analysis

2.1 Core SSR primer determination

A total of 102 polymorphic primers from 1 803 SSR basic primers were obtained by using the 5 varieties with distant relationship. Ultimately 20 primers were selected for further screening considering their stable repeatability and excellent polymorphism (Table 2). These 20 primers (or markers) belonged to 15 cotton chromosomes, and their polymorphism information content was different from each other. Twenty primers amplified a total of 116 alleles with 3～10 alleles for each and an average of 5.8. The PIC values ranged from 0.384 to 0.900 with an average of 0.716. The MI values ranged from 1.152 to 9.000 with an average of 4.374. Considering comprehensively the number of allele and PIC and MI values of each primer, we assumed that these 20 primers could be used as core primers for DNA fingerprinting analysis.

2.2 Genetic diversity analysis

Using the electrophoresis data of 20 pairs of primers, genetic similarity analysis was carried out for all materials. For 30 varieties, the pair-wise genetic similarity coefficients ranged from 0.19 to 0.88 with an average of 0.52 (Detail data not shown). Fig. 1 shows a dendrogram of 30 varieties based on the SSR data. Thirty materials were divided into 7 clusters (A, B, C, D, E, F and G) when adopting 0.52 as a threshold. Clusters A and F contained only one of two varieties, CIR12 and SGK958, respectively; clusters E and G contained 2 varieties; cluster D contained 4 varieties and clusters B and C contained 10 varieties. The system tree also indicated that five materials including Jinmian 36 (Deltapine), Xuzhou 142 (Stoneville), Simian 3 (Foster), CIR12 (Uganda) and Lumian 21 (Uganda) belonged to 4 clusters (C, D, A and B), respectively.

Table 2　Details of 20 pairs of primers with excellent polymorphism

Primer ID	Chr.	No. of alleles	PIC value	MI value
NAU1028	17	3	0.384	1.152
NAU3703	11	3	0.571	1.714
NAU5104	14	3	0.623	1.870
NAU0905	25	4	0.592	2.368
NAU2627	16	4	0.633	2.532
NAU1255	19	4	0.665	2.662
NAU2092	1	4	0.527	2.108
NAU3337	15	4	0.707	2.828
NAU4024	14	5	0.789	3.946
NAU5262	10	5	0.800	4.000
NAU3110	19	6	0.829	4.976
NAU2251	12	6	0.727	4.360
NAU1043	7	7	0.781	5.469
DPL0209	11	7	0.786	5.505
NAU1085	7	7	0.800	5.602
NAU3254	1	7	0.813	5.694
NAU2869	2	9	0.780	7.016
NAU3839	3	9	0.856	7.705
NAU4903	24	9	0.797	7.176
NAU2121	5	10	0.900	9.000

2.3 DNA fingerprinting of 30 varieties

The electrophoresis patterns of the 20 pairs of core primers showed that 4 of 30 varieties had their specific primers, by which each of them could be distinguished from any other variety. The variety CIR64 had two specific primers NAU4903 and DPL0209; the variety Liaomian 19 had two specific primers NAU2251 and NAU2092; the variety Simian 4 had one specific primer NAU4903; Xinqiu 1 had one specific primer NAU1043. For the other 26 varieties, they could be distinguished from any other variety by using the combinations of at least 2 pairs of primers. For example, primer combination of NAU2092/NAU1043 could distinguish Jimian958 from any other variety. Electrophoresis pattern of primers NAU2092 and NAU1043 for 30 varieties were shown in Fig. 2. The electrophoresis fingerprinting pattern of specific primers for 4 varieties and primer combinations for other 26 varieties were shown in Fig. 3.

2.4 Digital fingerprinting codes of 30 varieties

In order to identify the 30 varieties more conveniently and accurately, "0/1" binary data of 20 pairs of SSR primers were converted into decimal

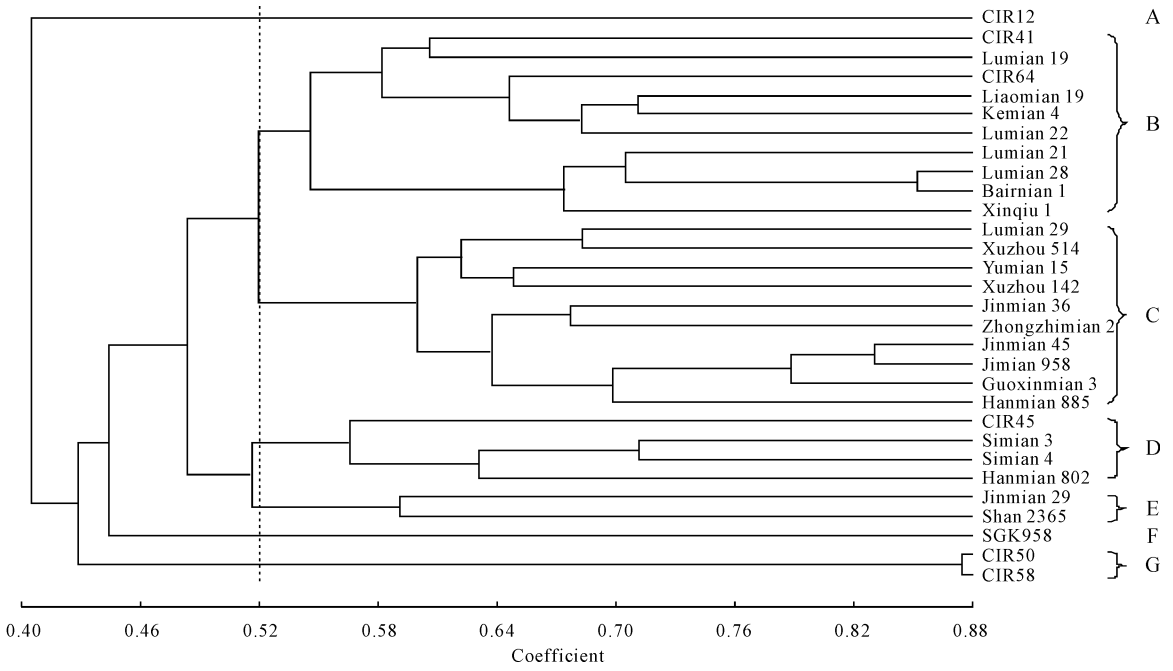

Fig. 1 Dendrogram of 30 Upland cotton varieties derived from SSR data using unweighted pair group of arithmetic means (UPGMA)

The scale is based on Jaccard's similarity coefficients

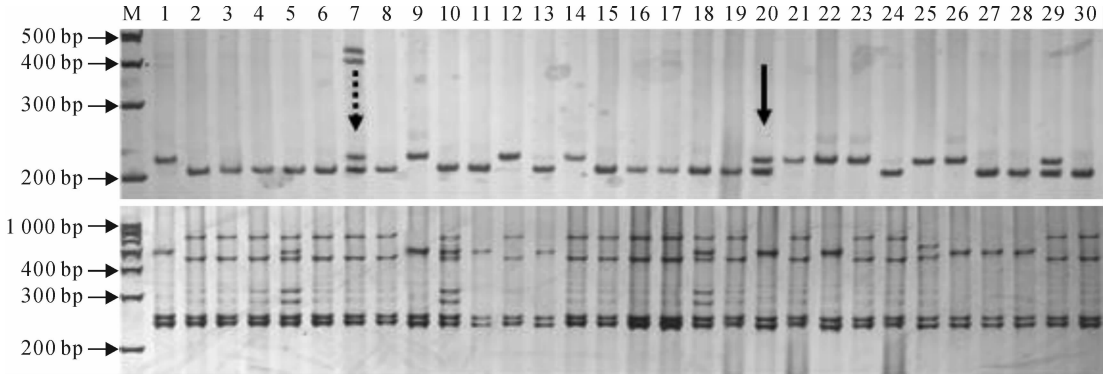

Fig. 2 Electrophoretic patterns of primers NAU2092 and NAU1043 PCR products for 30 Upland cotton varieties

1～30 corresponds to the material in Table 1, respectively; Upper for primer NAU2092 and lower for primer NAU1043; Dotted arrow marks variety 7, Liaomian 19, and solid arrow marks variety 20, Jimian 958

data. Assuming the most decimal digits as standard, if the digits are not enough for a certain material, "0" could be added in the front of the digital for the sake of achieving the same digits. Using decimal digital string from the electrophoresis patterns of the 20 core SSR primers to stand for the fingerprinting code of each material, fingerprinting codes changed simple and convenient that it improved the ability to distinguish one variety from the other varieties. Table 3 listed the decimal digital

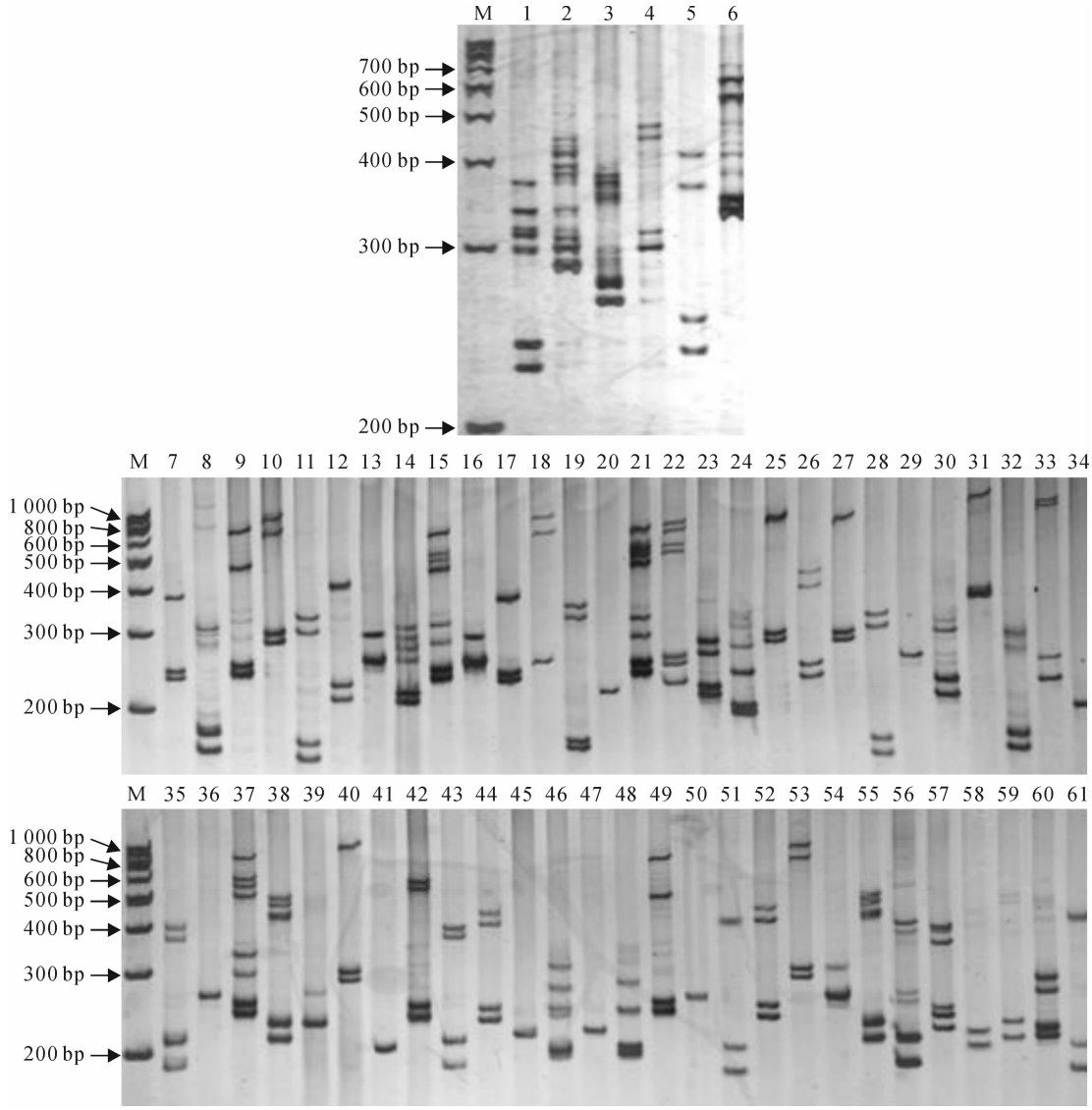

Fig. 3　Electrophoresis fingerprinting patterns of specific primers for 4 varieties and primer combinations for other 26 varieties

1,2. Specific primers NAU4903 and DPL0209 for CIR64; 3,4. Specific primers NAU2251 and NAU2092 for Liaomian 19; 5. Specific primer NAU4903 for Simian 4; 6. Specific primer NAU1043 for Xinqiu 1; 7,8. NAU1255/NAU2251 for CIR12; 9,10. NAU1043/NAU5104 for CIR41; 11,12. NAU0905/NAU2627 for CIR45; 13,14. NAU1085/NAU3110 for CIR50; 15,16. NAU1043/NAU1085 for CIR58; 17,18. NAU1255/NAU5262 for Lumianyan 19; 19,20. NAU0905/NAU2092 for Lumianyan 21; 21,22. NAU1043/NAU2121 for Lumianyan 22; 23,25. NAU3110/NAU4024/NAU5104 for Lumianyan 28; 26,27. NAU1028/NAU5104 for Lumianyan 29; 28,29. NAU0905/NAU1085 for Jinmian 29; 30,31. NAU2251/NAU3839 for Jinmian 36; 32,34. NAU2251/NAU5262/NAU2092 for Jinmian 45; 35,36. DPL0209/NAU1085 for Simian 3; 37,38. NAU1043/NAU2627 for Yumian 15; 39,40. NAU2869/NAU5104 for Shan 2365; 41,42. NAU2092/NAU1043 for Jimian 958; 43,44. DPL0209/NAU1028 for Hanmian 802; 45,46. NAU2092/NAU4024 for Hanmia 885; 47－49. NAU2092/NAU4024/NAU1043 for Xuzhou 142; 50,51. NAU1085/NAU3703 for Xuzhou 514; 52,53. NAU1028/NAU5104 for Baimian 1; 54,55. NAU1085/NAU2627 for SGK958; 56,57. DPL0209/NAU1255 for Guoxinmian 3; 58,59. NAU2092/NAU2627 for Kemian 4; 60,61. NAU3110/NAU3703 for Zhongzhimian 2

Table 3 Decimal digital fingerprinting codes of 30 varieties by electrophoresis of 20 core primers

Code	Variety	NAU1028-NAU3703-NAU5104-NAU0905-NAU2627-NAU1255-NAU2092-NAU3337-NAU4024-NAU5262-NAU3110-NAU2251-NAU1043-DPL0209-NAU1085-NAU3254-NAU2869-NAU3839-NAU4903-NAU2121
1	CIR12	4-4-2-10-12-10-02-10-04-25-50-42-24-098-050-005-330-198-166-0810
2	CIR41	4-3-5-10-12-05-01-10-27-06-50-42-68-013-050-026-330-198-166-0213
3	CIR45	4-3-3-05-03-05-01-10-27-25-13-21-68-098-050-005-330-062-166-0213
4	CIR50	4-3-3-10-12-05-01-01-04-06-63-21-68-013-005-026-330-325-085-0213
5	CIR58	4-3-3-10-12-05-01-01-04-06-13-21-95-013-005-026-330-325-085-0213
6	CIR64	4-3-3-10-12-05-01-15-31-06-63-21-68-127-127-127-251-477-249-1023
7	Liaomian 19	4-3-7-10-15-15-15-15-27-25-63-63-68-013-127-127-330-479-166-1023
8	Lumianyan 19	4-3-2-10-12-10-01-01-27-06-50-21-68-013-050-005-330-062-166-0213
9	Lumianyan 21	4-3-2-10-12-05-02-01-04-06-50-21-24-013-050-005-330-198-166-0213
10	Lumianyan 22	7-3-2-10-12-15-01-15-31-31-63-21-95-013-050-005-330-254-166-1023
11	Lumianyan 28	2-3-2-10-12-05-01-01-04-25-13-21-24-013-050-005-330-198-166-0213
12	Lumianyan 29	4-3-6-10-12-05-02-01-27-25-50-21-68-013-005-005-330-198-166-0810
13	Jinmian 29	7-3-3-05-00-05-01-01-27-06-13-21-24-098-050-026-330-477-166-0810
14	Jinmian 36	4-3-2-10-12-05-02-10-27-06-50-42-68-013-050-005-330-450-166-0810
15	Jinmian 45	4-3-6-10-12-05-01-01-27-25-50-21-68-013-050-005-330-325-166-0810
16	Simian 3	4-3-7-10-12-05-01-10-27-25-13-21-68-098-005-005-330-198-166-1023
17	Simian 4	4-3-3-10-12-05-01-10-27-25-13-21-68-098-050-005-330-325-394-0810
18	Yumian 15	4-3-3-10-15-15-01-01-27-31-50-21-95-013-127-127-330-101-166-0810
19	Shan 2365	4-3-6-10-03-05-01-10-27-06-13-21-68-013-050-026-069-479-166-0810
20	Jimian 958	4-4-5-10-12-05-03-10-27-25-50-21-24-013-050-005-330-325-166-0810
21	Hanmian 802	2-3-5-10-12-05-02-10-27-06-13-21-68-098-050-005-069-198-166-0810
22	Hanmian 885	7-3-2-10-12-05-02-01-31-06-50-21-24-013-050-005-330-325-166-0810
23	Xuzhou 142	4-3-2-10-12-05-02-01-04-25-50-21-68-013-050-026-330-101-166-0810
24	Xuzhou 514	4-4-3-10-00-05-01-01-27-06-50-21-68-013-005-026-330-198-166-0810
25	Xinqiu 1	4-3-2-10-12-05-02-01-27-25-50-21-44-013-050-026-330-450-166-0213
26	Baimian 1	2-3-3-10-12-05-02-01-04-25-13-21-24-013-050-005-330-198-166-0213
27	SGK958	4-7-2-10-15-10-01-10-27-25-13-21-24-013-005-005-069-485-166-0213
28	Guoxinmian 3	4-3-6-10-12-15-01-10-27-25-50-21-24-098-050-005-330-325-166-0810
29	Kemian 4	4-3-7-10-00-05-03-15-27-31-63-21-68-013-050-127-330-198-166-0213
30	Zhongzhimian 2	4-4-6-10-12-05-01-10-27-06-13-21-68-013-050-005-330-198-166-0810

fingerprinting codes of 30 varieties by electrophoresis patterns of the 20 core SSR primers.

3 Discussion

Previous studies showed that among the molecular markers, SSR marker could efficiently and effectively reveal DNA fingerprinting of crop germplasm collections[29-31]. The SSR primers can be divided into three types: the first one is the primers within gene, and they can be used to identify genes for some important agronomic traits; the second type is the SSR markers linked tightly with genes for some traits, and they can be used to identify genes for disease-resistant and stress-resistant, as well as some important quality traits; the other type is the SSR makers linked non-tightly with genes for any of traits. When DNA fingerprinting is used to identify varieties and their purity, priority should be given to the frontier two types of SSR primers, followed by the third type. In the past, the basic primers used for analyzing cotton genetic diversity and fingerprinting are relatively less, and most of them belong to the third type. Therefore, the polymorphism among materials is not high, and even if polymorphism is existed, these polymorphisms have no corresponding relationship with phenotypic differences in materials. In this study, Referring to the published cotton interspecific and intraspecific genetic maps[18,20,22], the SSR primers (or markers) were selected from cotton 26 chromo-

somes, and these primers mainly belong to the third type. Because they came from genetic maps, the polymorphism among germplasms was relatively high. At the same time, we also focused on the selection from the reported SSR markers, which were co-separated or closely linked with genes for cotton important traits[16-17,19,21,23]. These primers mainly belong to the first or second types. Therefore, comparing with the previous studies, the basic primers in this study can effectively be used for polymorphism scanning of cotton germplasms.

In recent years, cotton DNA fingerprinting has been widely reported. Guo *et al*[32] constructed DNA fingerprinting of 9 main Upland cotton varieties in China using Random Amplified Polymorphic DNA (RAPD) marker. Song *et al*[33] distinguished 8 cotton varieties from each other using 26 pairs of Amplified Fragment Length Polymorphism (AFLP) primers. Wang *et al*[34] obtained the DNA fingerprinting of brown cotton "Three Lines" (sterile, maintainer and restorer) and their hybrid F_1 using AFLP markers. These studies fully reflected that applying molecular markers for cotton variety identification had a huge advantage. However, some studies are in the early stages of molecular marker development, and the experimental materials for constructing fingerprinting are mainly confined in the target materials without considering more background materials. In the process of construction and application of DNA fingerprinting, the problem highly concerned with breeders is whether the fingerprinting established by limited experimental materials could ensure the "specific" or "uniqueness" of variety. The substance of this matter involves the probability of exactly same fingerprinting occurring in all the genotypes within the scope of the same species. Because the number of allele of primer amplification will increase with an increase in experimental materials, using limited experimental materials for fingerprinting analysis might lead to a decrease in primer polymorphism information, and thus the specificity of target material map would be affected. If the number of other background materials is increased, some of specific primers of the target material would probably not be specific. The limited genetic diversity of cultivated Upland cotton has been observed previously[35-37]. In this study, however, 30 Upland cotton varieties are from multiple pedigrees. These materials are not only target materials but also background materials. Because the genetic basis of these materials was relatively expansive, it would be beneficial in obtaining the primers with rich polymorphism, which would ultimately improve the DNA fingerprinting with respect to "specific" or "uniqueness" of varieties.

The core primers have the proficient potential to differentiate among cotton cultivars from different pedigrees and regions. In addition, implementation of potential core primers would turn to be a helpful preliminary tool for cotton breeders to successfully pick up genetically more distant parents for hybridization programs[38]. In this study, 5 varieties with further relationship, Jinmian 36 (Deltapine pedigree), Xuzhou142 (Stoneville pedigree), Simian 3 (Foster pedigree), CIR12 (Uganda pedigree) and Lumian 21 (Uganda pedigree) were selected to screen all primers in order to obtain the polymorphic core primers. Ultimately 20 of 1 803 pairs of primers were confirmed as core primers with stable repeatability and rich polymorphism. The dendrogram in Fig. 1 showed that all 30 materials were divided into 7 clusters when adopting 0.52 as a threshold. Especially the five materials, Jinmian 36, Xuzhou 142, Simian 3, CIR12, and Lumian 21 were respectively distributed to four clusters, which confirmed that the selection of the five materials for obtaining polymorphic core primers was feasible.

Presently, SSR fingerprinting database of the main crops such as tomato[4], rice[6] and wheat[7] have already been constructed. There is a need of large database for a set of core primers with comprehensive good characteristics of high polymorphism, stability and repeatability. In a research of cotton DNA fingerprinting, Pan *et al*[12] used 12 accessions with significantly different phenotypes and genetic backgrounds as panel germplasms for screening 5 914 SSR primers. The results showed

that 319 pairs of polymorphic SSR primers with suitable amplification and clear bands could be regarded as core primers for evaluating cotton germplasms and depicting fingerprinting. Of which, 277 pairs of primers could identify different Upland cotton varieties. In addition, 13 primer pairs with polymorphism in all three species of Upland cotton, Sea Island cotton and Asiatic cotton were recommended as the first-choice primers for molecular fingerprinting and germplasm identification. In this study, we also performed amplification among experimental materials using nine of 13 primer pairs recommended by Pan *et al*[12], and the results showed that only NAU1085, NAU2251 and DPL0209 had polymorphism, which suggested that the core primers would be adjusted according to concrete experimental materials with the further development of fingerprinting. The core primers obtained in this study is a good supplement of previous study. Our research could provide some important references for constructing standard DNA fingerprinting database of cotton. In recent years, cotton genome research has made rapid development, and genome sequencing of *G. raimondi* has already been completed[39]. With the progress in genome sequencing of Upland cotton, more functional and intron markers will be expected to change into core primers applied for cotton germplasm identification and fingerprinting analysis.

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