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Genetic diversity analysis of various red spider mite-resistant upland cotton cultivars based on RAPD

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This study is carried out to analyze the genetic diversity of red spider mite-resistant cotton (*Gossypium hirsutum*) cultivars that are applied in cultivar identification and breeder's right protection of cottons. The genomic DNA was used as template and random primers were used to analyze the genetic diversity of 21 accessions of *Gossypium hirsutum* by RAPD-PCR. Among the 100 primers screened, 20 primers could generate 176 fragments, 96.02% of which were polymorphic ones. The similarity coefficient of cultivars was between 0.2273 - 0.9773. The average values of effective number of alleles, Nei's gene diversity and Shannon's information index were 1.7391, 0.4017, and 0.5773, respectively. Cluster analysis based on UPGMA revealed that 21 cultivars could be divided into three groups. The analysis revealed that it was corresponding to the geographical distribution and most of the lines had a wide genetic base.

Key words: Cotton, red spider mite, RAPD, genetic diversity, cluster analysis.

INTRODUCTION

Red spider mite (*Tetranychus* sp.), which is one of the four most popular cotton pests in the world, mainly damages the leaves in middle part of the cotton plants and to absorb the juice on the back of the leaves, resulting in red leaves of cotton plants. If the damages were serious, the leaves will drop. Moreover, vast abscission of cotton buds and bolls will be caused as well. Once the cotton plants are damaged by red spider mite, production of cotton will be reduced by 18 - 57% (Canerday and Arant, 1964; Roussel et al., 1951; Schuster and Jenkins, 1975). Selective breeding and application of red spider mite-resistant cotton cultivars is the most effective and economical methods for

prevention and control of red spider mite (Wang et al., 2001; Zhang, 1986, 1993). Presently the known red spider mite-resistant genetic resources mainly exist in some wide cottons, semi-wide upland cottons, and *Gossypium barbadense*. Thus, genetic transference of red spider mite-resistant gene to the wide-used upland cotton cultivars through distant hybridization is of great practical significance. Since 1970s, our country has successfully cultivated several decades of red spider mite-resistant cottons, which offer basic resources and data for further researches on breeding of mite-resistant cotton cultivars. However, up till now, there are no systematic researches on genetic composition of these mite-resistant cotton cultivars and the genetic relationship among these plants, which to some extent acts as an obstacle for further and effective application of these valuable materials.

Random amplified polymorphic DNA (RAPD) is a DNA molecular marker technology initiated by Williams et al. (1990), which has advantage in high-efficiency, small usage of samples, sharp sensibility and easy-detection. To control the experimental condition strictly, a good

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Abbreviations: RAPD, Random amplified polymorphic DNA; CTAB, cetyl trimethyl ammonium bromide; PCR, polymerase chain reaction.

Table 1. Names and origins of tested cottons.

Code	Accession	Origin	Code	Accession	Origin
1	JCG68	USA	12	Heze839(2)-7	Shandong
2	Liao 96-23-30	Liaoning	13	Lu11#	Shandong
3	Kashi 7736	Urumqi Xinjiang	14	Ji 475	Hebei
4	90-658-18	Urumqi Xinjiang	15	Mianyang 4176	Sichuan
5	Kuche T94-4	Urumqi Xinjiang	16	Tamcot GCNH	USA
6	Kuche 9100A-6	Urumqi Xinjiang	17	AL-SEEMI241	USA
7	Kuche 9100A-11	Urumqi Xinjiang	18	Chuan 45	CRI.CAAS
8	Kuche 9100A-13	Urumqi Xinjiang	19	Chuan 98	CRI.CAAS
9	Uzbek 3#	Uzbek	20	9809	CRI.CAAS
10	Zhong 99	CRI.CAAS	21	9812	CRI.CAAS
11	Line1#	Unknown			

CRI.CAAS: Cotton Research Institute, China Acad. Agric Sciences. ICI.SAAS: Industrial Crop Research Institute, Sichuan Acad. Agric Sciences.

repeatability of the result was realized (Williams et al., 1990). Thus, it has been widely used in many perspectives including systematic of animals, plants, human beings and microorganism, gene mapping, line identification, medical diagnosis, genetic map construction, and genetic polymorphism.

This study selected the mite-resistant cotton cultivars preserved by Cotton Research Institute of Chinese Academy of Agricultural Sciences and the red spider mite-resistant upland cotton cultivars bred in Sichuan as the research materials. By using RAPD, fingerprinting of red spider mite-resistant cotton cultivars is built. Besides, genetic diversity of mite-resistant cotton resources in our country is analyzed based at molecular level, which is expected to offer theoretical basis for the effective and complete utilization of red spider mite-resistant resources.

MATERIALS AND METHODS

Materials

The screening study was undertaken at the farm of ICI.SAAS in 2007 to seek promising genetic types of cotton with resistance to spider mites, particularly the two-spotted mite, *Tetranychus telarius* (L.). Twenty-one (21) mite-resistant upland cotton cultivars (Table 1) from 81 have been screened to date, which are widely used cotton breeding and conducted an RAPD analysis.

DNA extraction

For each material, 10 cotton plants were selected at random from the field and their tender leaves (foliar age less than one week) were used for DNA extraction. The method of extraction was based on Doyle's (1990) cetyl trimethyl ammonium bromide (CTAB) while some modification was made and the relevant details were stated in the previous report (Zhang et al., 2008). Regarding the test purity and density of DNA, 0.8% agarose gel and BioSpec-mini spectrophotometer (Shimadzu, Japan) were employed. Finally, the

DNA was diluted to 60 ng/ μ l, preserved at -20°C.

RAPD analysis

Twenty microliters (20 μ l) system consists of 60 ng DNA, 2 mmol/L dNTPs 2 μ l, 10 μ mol/L random primer 2 μ l, 10 \times buffer 2.0 μ l (including Mg²⁺), and 1.0 U Taq enzyme. Finally, hyper pure water was added to complement the system to 20 μ l. According to the above reaction system, the mixed liquor was prepared and then covered with mineral oil. PCR amplification was conducted through PTC-100TM. And the condition for such amplification was 94°C for 6 min one circulation, 94°C for 1 min, 36°C for 1 min, 72°C for 2 min 42 circulations. After the end of reaction, the mixed liquor was reserved at 4°C until electrophoresis of 1.0% agarose gel including 0.5 μ g/ml EB, which lasted for 1.0 h. Each random primer would be repeatedly tested for 2 - 3 times.

Data analysis

RAPD band is dominant marker. According to the electrophoresis result of polymerase chain reaction (PCR) amplification products, in the mobility position of gel, these with DNA band was marked as "1", while marked "0" if it is with no DNA band. Cluster analysis was then conducted by using NTSYS-pc2.10 analytical software, Jaccard similarity coefficient and UPGMA (Unweighted Pair Group Method with Arithmetic Mean). Through employing POPGEN 1.31 software, percentage of polymorphic Loci (PPL) and Shannon Information Diversity Index were calculated. Moreover, Nei's Gene Diversity (He) was computed, while the number of alleles (na) and the effective number of alleles (ne) were observed.

RESULTS AND ANALYSIS

Result of RAPD-PCR

By using three accessions with large phenotypic differences, 30 primers were screened from 100 primers. The selected primers possessed clear bands and good

polymorphisms for diversity analysis of red spider mite-resistant cottons. After the elimination of illegible primers with poor amplification results, finally 13 primers were left for the research of genetic diversity of 21 materials (Table 2). Totally 176 RAPD bands and 169 polymorphic bands were generated. Polymorphic rate reached as much as 96.02% and the sizes of bands varied from 300 - 2000 bp (Figure 1). The number of RAPD band generated by each primer was 5 - 12, realizing an average number of 8.5. Among the primers, OPH6, OPN14, OPN19 generated most of the bands of 12 polymorphic bands, respectively, while primers of OPG6 and OPH8 only produced 5 polymorphic bands, which was the least.

Analysis of genetic diversity of red spider mite-resistant cottons based on RAPD

The effective number of alleles (n_e) and Nei's genetic diversity index (H_e) are the two most frequently used indices for the measurement of genetic variation, both of which are of great genetic significance. Shannon information index itself has no genetic meaning, but it facilitates comparison with similar studies. By using software packages POPGEN32, effective number of alleles (n_e), Nei's genetic diversity index (H_e) and Shannon information index (I) of the red spider mite-resistant cottons were computed. The results revealed that the average values of number of alleles, effective number of alleles, Nei's genetic diversity (H_e) and Shannon information index were 1.9602, 1.7391, 0.4017 and 0.5773, respectively. Regarding each loci, there was significant difference in the degree of genetic diversities. Maximum value of effective number of alleles was 1.9988 and the minimum value was 1.0494. Maximum value of Nei's genetic diversity index was 0.5773 and minimum value was 0.047. Concerning Shannon information index, its maximum and minimum value separately were 0.6929 and 0.1136. All these proved rich genetic diversity of the 21 red spider mite-resistant cottons.

UPGMA cluster analysis of 21 red spider mite-resistant cottons based on similarity coefficient

Taking the fragments data of 176 loci of 21 red spider mite-resistant cottons as the original matrix, along with the obtained 210 cultivars similarity coefficients, it is known that amplitude of cultivars similarity coefficients varied a lot and between 0.2273 - 0.9773. The similarity coefficient of '9809' and '9812' was the largest, which was 0.9773, whereas, that of "Mianyang 4176" and 'Liao 96 - 23 - 30' was 0.2273, which was the smallest.

Twenty one (21) red spider mite-resistant cottons can be divided into three groups based on the threshold value of 0.69 (Figure 2). Group I covered most of the accessions (13 accessions), including the four accessions selected

from the after-generation of cross-fertilized breed of island and upland cotton by Sichuan Academy of Agricultural Sciences, three accessions from Kuche, Xinjiang, two accessions from Shandong and the rest four accessions. Number of accessions in Group III ranked the second, covering 5 accessions. The rest three accessions (Ji 475, Mianyang 4176, AL-SEEMI241) constituted Group III. Among the accessions in Group I, most of the materials had genetic relationship with Deltapine Cotton. The materials in Group II were almost originated from 39° Noth-42° North. Concerning the materials in Group III, particularly Mianyang 4176, it was originated from the cross-fertilized breed of Mianyang 1386 and Jihe 3016, while it also had some genetic relationship with Ji 475.

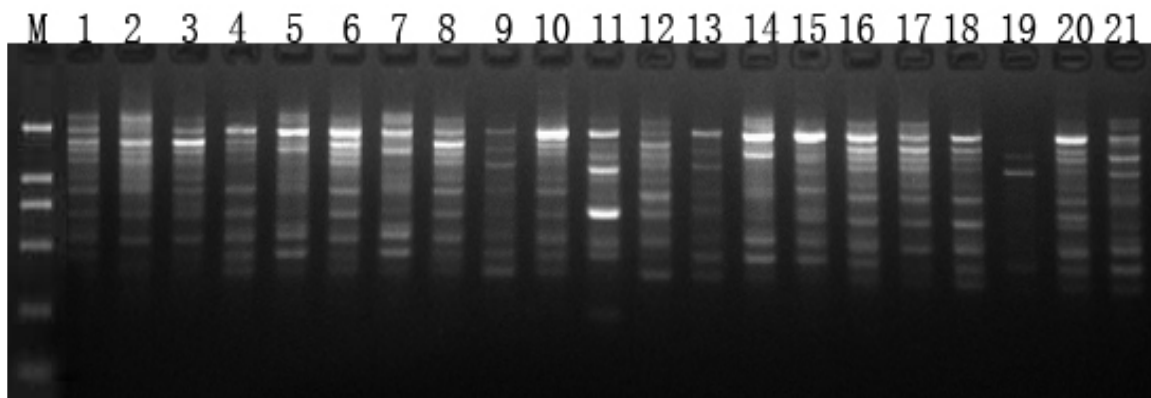
DISCUSSION

Analysis of genetic diversity of crops resistant materials is an important part of the researches on genetic resources. Also, it is induced to guide the crop breeding and work out rational breeding programs. Random amplified polymorphic DNA (RAPD) is characterized in flexibility and convenience. So long as the experimental conditions are strictly controlled, a good and repeated result will be gained, which will also play a significant role in the researches on genetic diversity of animals and plants. RAPD also was used to reveal the genetic relationship of elite commercial cotton varieties with some standard "Coker" varieties and the diploid *G. arboreum* L. var. Ravi (old world cotton) (Iqbal et al., 1997). In China, thirty varieties belonging to *Gossypium hirsutum* L. were analyzed by RAPD. This revealed that the intervarietal genetic relationship of several varieties was related to their pedigrees of the parent and most of the varieties had a narrow genetic base (Bie et al., 2001). Guo et al. (1999) identified the genetic variation of 25 verticillium wilt cottons based on RAPD, which revealed the genetic actuality of exiting verticillium wilt breeds in China. Zhu et al. (2002) conducted random amplified polymorphic DNA analysis of transgenic cotton harboring single and double-gene during their researches on genetic variation of cotton genetic resources originated from different places. The research findings coincided with Guo et al. (1997) analytic result of assessment on the genetic diversity of cottons in China based on RAPD. There were great differences among different accessions owing to the varied genetic background, while upland cottons have a narrow genetic base. From the research result in this study, it is known that red spider mite-resistant upland cottons have rich genetic diversity. Thus, corresponding RAPD, fingerprint files can be established so as to offer scientific base for the protection of these valuable resources and improved breeds, molecular identification of lines, as well as cultivation and purity testing of red spider mite-resistant cottons, etc.

Selective breeding and application of red spider mite-

Table 2. RAPD primers used in analysis of genetic diversity of red spider mite-resistant cottons and their amplification results.

Primer	Sequence of primer	Number of bands	Number of polymorphic bands
OPA5	AGGGGTCTTG	8	8
OPG6	GTGCCTAACC	5	4
OPG16	AGCGTCCTCC	8	6
OPG17	ACGACCGACA	9	9
OPH6	ACGCATCGCA	12	12
OPH7	CTGCATCGTG	10	10
OPH8	GAAACACCCC	5	5
OPH11	CTTCCGCAGT	9	9
OPH12	ACGCGCATGT	9	6
OPH14	ACCAGGTTGG	8	8
OPM3	GGGGGATGAG	9	9
OPN10	ACAACCTGGGG	7	7
OPN11	TCGCCGCAA	9	9
OPN13	AGCGTCACTC	7	7
OPN14	TCGTGCGGGT	12	12
OPN19	GTCCGTA CTG	12	12
OPN20	GGTGCTCCGT	11	11
OPP2	TCGGCACGCA	9	8
OPS1	CTACTGCGCT	9	9
OPS15	CAGTTCACGG	8	8
Total		176	169

**Figure 1.** The results of amplification by OPN14 (M, Marker D2000, Lanes 1-21 represents 1-21 materials respectively. For details, please refer to Table 1).

resistant cottons is the most effective method for prevention and control of red spider mite. Therefore, researches on genetic diversity of different lines will not only offer reference to the constitution of selective breeding of new accessions and rational distribution of accessions, but also provide some predictive guidance to parent selection, degree of genetic variation, and forecast of cross-fertilized advantages. The research results indicate that cluster analysis of different genetic resources and

materials is not simply based on the resistant degree of red spider mite. Although the study introduced samples failed to resist red spider mite as control group, the cluster result was inconsistent with the resistant expression of different materials but in high consistency with the corresponding originations of pedigree. The fact revealed rich genetic diversity of the tested samples. Thus, there exists big difference among various red spider mite-resistant, which is of great practical potential for breeding.

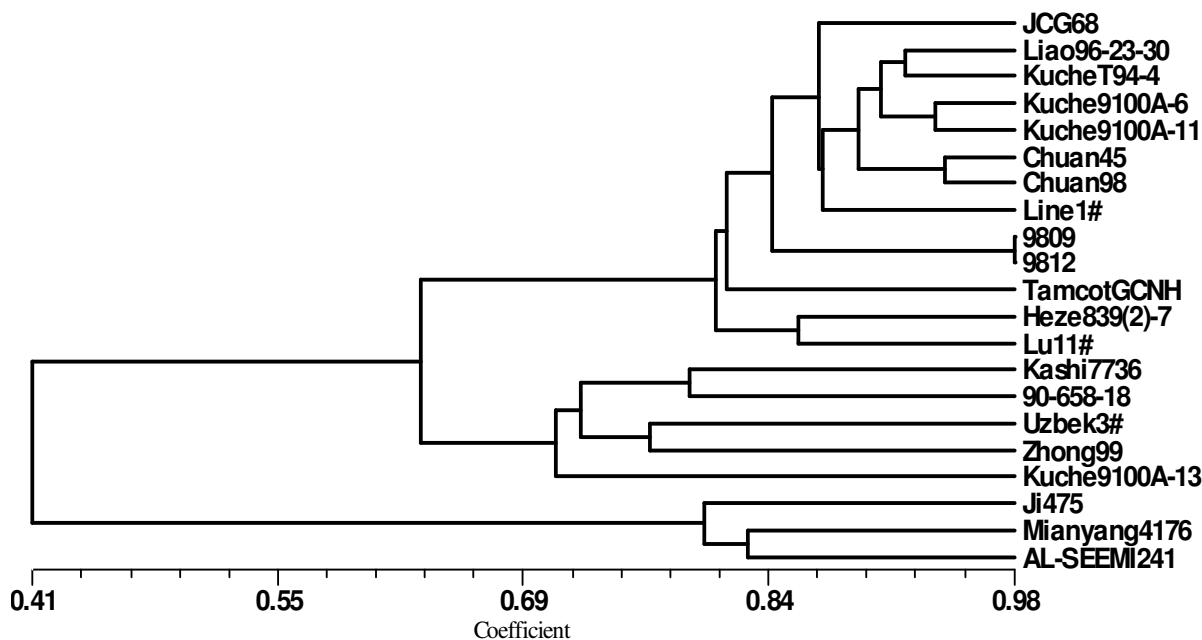


Figure 2. Dendrogram of 21 experimental materials based on RAPD.

Zhang (1992) argued that by collecting the existing cottons and selecting various red spider mite-resistant upland cottons from historic lines, the demand of material for the breeding of red spider mite-resistant cotton would be satisfied. However, presently, the red spider mite-resistant genetic resources are mainly distributed in wide cottons, upland cotton of semi-wide species, and *G. barbadense* (Maxwell and Jennings, 1980; Schuster and Jenkens and Jenkens 1972; Zhang, 1992). Through distant hybridization, red spider mite-resistant gene will be transferred to upland cottons and the genetic background of upland cottons will be enriched. After many years' of breeding practice, breeders have made great progress in developing the red spider mite-resistant genetic resources and new cultivars. Zhang (1993) screened some red spider mite-resistant materials from the cross-fertilized breeds of *G. hirsutum* × *G. barbadense*, *G. arboreum* × *G. hirsutum*, *G. anomalum* × *G. arboreum* × *G. hirsutum*. Wang et al. (1991, 2001), who came from Sichuan Academy of Agricultural Sciences, succeeded in cultivating red spider mite-resistant germplasm Chuan 98 (Dong et al., 2001) and new red spider mite-resistant, bumper, high-quality and disease-resistant cultivars such as 9812, 98-19 and red spider mite-resistant Chuan 45.

After RAPD analysis of the red spider mite-resistant materials cultivated by Sichuan Academy of Agricultural Sciences, it was discovered that red spider mite-resistant cottons were mainly originated from hybrid progeny of *G. barbadense* and upland cotton, which formed a large cluster with the three accessions from Kuche, Xinjiang, two accessions from Shandong and the other four accessions. Moreover, pedigree analysis showed that most of

the materials had genetic relationship with Deltapine Cotton, which has further proved the reference value of RAPD result. Besides, Ji 475 and Mianyang 4176 jointly formed a cluster. After investigating, it is found that Mianyang 4176 originated from selective breeding of the hybrid progeny of Mianyang1386 and Jihe3016, which showed that Ji 475 may have the same origination with Jihe 3016 and had genetic relationship with Mianyang 4176. Analysis on the cluster of materials and genetic relationship should be based on the similarities among genetic materials. Moreover, in this study, RAPD analysis also revealed that materials selected from hybrid progeny of *G. barbadense* and *G. hirsutum* also maintained rich diversity. Thus, it is proved that to create resources with rich genetic background through distant hybridization of island cotton and upland cotton is a feasible way, which can fully make use of the resistant gene to red spider mite and verticillium in island cotton so as to extend the genetic background of upland cottons. All these will lay a form foundation for the selective breeding of improved cottons.

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