Front. Agric. China 2007, 1(2): 129–135
DOI 10.1007/s11703-007-0023-x

RESEARCH ARTICLE

WANG Xingfen, MA Jun, YANG Shuo, ZHANG Guiyin, MA Zhiying

Assessment of genetic diversity among Chinese upland cottons with Fusarium and/or Verticillium wilts resistance by AFLP and SSR markers

© Higher Education Press and Springer-Verlag 2007

Abstract Genetic diversity among 95 Chinese upland cottons with Fusarium and/or Verticillium wilts resistance was estimated using Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) markers. Twenty *Eco*RI-*Mse*I AFLP and 19 SSR primers with polymorphism were selected to perform the fingerprinting. The results showed that 20 AFLP primer pairs produced a total of 1 480 major bands among 95 genotypes, and 214 were polymorphic bands. The number of total bands per primer pair ranged from 47 to 109, with an average of 74.0. The polymorphism information content (*PIC*) values for the 20 primer pairs varied from 0.01 (E-ACT/M-CAT) to 0.24 (E-ACA/M-CTA), and the average value was 0.09. Nineteen SSR primers generated 89 DNA bands, of which 61 were polymorphic. The total number of alleles per locus varied from 3 to 8, with an average of 4.7. The average *PIC* value for the SSR amplification products was 0.69. Genetic similarity estimates for the entire data set ranged from 0.978 to 0.998 based on AFLP and SSR bands. It was proved that the close genetic relationship and narrow genetic diversity existed in the tested cultivars. The clustering patterns could not be correlated to the geographic origin, the pedigree and common parentage of the cultivars.

Keywords Amplified Fragment Length Polymorphism (AFLP), cluster analysis, cotton (*Gossypium hirsutum* L.), genetic relationship, Simple Sequence Repeat (SSR)

1 Introduction

Cotton is a pivotal cash crop in China with annual lint production of about 6 million tons. There are 4 major cotton-growing regions of Yellow River Valley (YRV), Yangtze River Valley (YzRV), Northwest Inner Land (NwIL) and North Very Early Maturity (NVEM). More than 95 percent of cotton acreage devoted to *Gossypium hirsutum* L.. During the cotton growing period, Fusarium wilt and Verticillium wilt do serious harm to cotton, and greatly affect its production and fiber quality worldwide. It has been confirmed that the most effective and economical pathway to control the two diseases is selection and utilization of the highly resistant cultivars (Verhalen, 1971; Roberts and Staten, 1972).

In China, cotton resistant breeding for the two diseases may date back to 1950s. Since then, various research institutes have released over one hundred cultivars through intensive breeding programs. From 1950s to 1970s, pedigree selection was mainly performed, thereafter, hybridization breeding dominated in breeding program. To date, pedigree relationships among some cultivars were studied (Feng et al., 1996), but little information was available on the genetic relationship and composition of various resistant cultivars at DNA level. Thorough understanding of genetic relationship and diversity of cotton disease-resistant germplasms is critical for conservation of genetic resources and development of breeding strategies that are involved in parental selection to maximize genetic improvement and enhancement. More accurate and complete descriptions of existing cotton cultivars and their patterns of genetic diversity could facilitate introgression of diverse germplasm into current cotton genetic base (Satyavathi et al., 2006).

In recent years, some reports have described the diversity and relationship of cotton cultivars or species as revealed by molecular marker techniques, such as RFLP, RAPD, AFLP and SSR (Wendal et al., 1992; Brubaker et al., 1994; Multani and Lyon, 1995; Tatineni et al., 1996; Iqbal et al., 1997, 2001; Small et al., 1999; Liu et al., 2000; Abdalla et al., 2001; Rana and Bhat, 2002; Rahman et al., 2002; Rana et al., 2005; Zhang et al., 2005). AFLP and SSR techniques have been shown to be powerful tools for studying genetic diversity and variation in plants (Incirli and Akkaya 2001; Kim et al., 2002; Mellish et al., 2002; Medini et al., 2005). Moreover, the two

Received March 26, 2007; accepted April 4, 2007

WANG Xingfen, MA Jun, YANG Shuo, ZHANG Guiyin, MA Zhiying (✉)
Key Laboratory of Crop Germplasm Resources of Hebei Province, Agricultural University of Hebei, Baoding 071001, China
E-mail: mzhy@hebau.edu.cn

techniques provide an accurate way to fingerprint closely related cultivars for identification, cultivar purity maintenance and for intellectual property protection of newly bred cultivars (Soleimani et al., 2002). However, no study has been reported on genetic diversity of China-bred cottons resistant to Fusarium and Verticillium wilts using AFLP and SSR techniques.

The objective of the present study is to assess the genetic diversity among the existing Chinese cottons with Fusarium and/or Verticillium wilts resistance by using AFLP and SSR markers, to compare the genetic similarity obtained from AFLP and SSR, and to facilitate the use of germplasms in breeding of disease-resistant cottons.

2 Materials and methods

2.1 Plant materials

According to cotton-growing regions, geographic origin and released time, 95 China-bred cultivars with Fusarium and/or Verticillium wilts resistance from 1950s to 1990s were used in the study (Table 1). These cultivars were non-transgenic insect-resistant ones and from the YRV, YzRV, NwIL and NVEM cotton-growing regions. Since cotton breeding was conducted mainly in the second half of the 20th century, most of the cultivars tested were registered within the past 40 years. Because of a rapid increase in breeding efforts in 1980s and 1990s, a large amount of cultivars were taken from these time periods (Fig. 1). All of the materials were provided by the Cotton Genetics and Breeding Institute of Agricultural University of Hebei, and by the Cotton Research Institute of Chinese Academy of Agricultural Sciences (CRI, CAAS).

2.2 Methods

2.2.1 DNA isolation

Germinated cotton seeds from each genotype were cultured in the growth chamber at 25–28°C for about one week. Total genomic DNA was extracted using about 3 g of fresh cotyledon tissue from 15–20 plants of each genotype according to Paterson et al. (1993) Protocol. DNA concentration was estimated using a Beckman DU800 spectrophotometer as well as by visual comparison to the known concentration of lambda DNA marker.

2.2.2 AFLP analysis

AFLP procedure was performed as described by Vos et al. (1995) with some modifications (Zhao et al., 2002). Genomic DNA (450 ng) was digested for 2 h at 37°C, with 3 U *Eco*RI (New England Biolabs, Beverly, Mass.) and 3 U *Mse*I (New England Biolabs, Beverly, Mass.) in 20 µL volume. Five microliters of the adapter-ligation solution (1.0 µL 50 pmol/µL *Mse*I adapter, 1.0 µL 5 pmol/µL *Eco*RI adapter, 1.5 U T_4 DNA ligase (Promega, Madison, Wis.), 0.5 µL T_4 buffer, 1.8 µL 10 mmol/L ATP and 0.2 µL ddH_2O) was added to the digested products and the reaction was allowed to take place for 10 h at 37°C. Five microliters of ligation products from a 1:10 dilution were used for PCR pre-amplification with primers carrying non-selective nucleotide. PCR cycles were performed at 95°C for 2 min, 95°C for 30 sec, 56°C for 30 sec and 72°C for 1 min in total 30 cycles, then 72°C for 10 min on a PTC-100 thermal cycler. The pre-amplification products were diluted 10-fold in 1 × TE and used as a template for selective amplification. One hundred of *Eco*RI:*Mse*I primer combinations randomly consisted of 10 *Eco*RI primers (including E-AAC, E-AAG, E-ACA, E-ACC, E-ACG, E-ACT, E-AGC, E-AGG, E-CGG and E-GGA) and 10 *Mse*I primers (M-CAA, M-CAC, M-CAG, M-CAT, M-CTA, M-CTC, M-CTG, M-CTT, M-GGA and M-TAT). The following PCR reactions were done at 95°C for 2 min, 95°C for 50 sec, 65°C for 40 sec, 72°C for 1 min, followed by 12 cycles with a 0.7°C decrease in annealing temperature in each cycle, and then it reached the optimal annealing temperature of 56°C. Thirty-one cycles were done at 95°C for 50 sec, 56°C for 40 sec, 72°C for 1 min, and then 72°C for 10 min to complete the selective amplification.

2.2.3 SSR analysis

For SSR analysis, 40 primers were selected for amplification. Genomic DNA concentration was adjusted to 20 ng/µL. Each 10 µL amplification reaction contained 40 ng of gDNA, 1 µL 10 × PCR buffer (including Mg^{2+}), 0.8 µL 2.5 mmol/L dNTP, 0.35 µL 10 ng/µL Forward Primer, 0.35 µL 10 ng/µL Reverse Primer, 0.8 unit of *Taq* DNA polymerase (TaKaRa, Dalian, Liaoning). PCR amplification was carried out under the following conditions: 95°C for 5 min, 30 cycles at (94°C for 45 sec, 55°C for 45 sec, 72°C for 1 min), 72°C for 10 min.

2.2.4 Electrophoresis

The amplified samples for AFLP and SSR assays were mixed with 5–10 µL loading buffer (98% formamide, 10 mmol/L EDTA, 0.025% xylene cyanol, and 0.025% bromophenol blue), heated at 95°C for 10 min, and then quickly chilled on ice for electrophoresis. PCR products were separated on 6% denaturing polyacrylamide gelswith a constant power (80 W) in 1×TBE running buffer, and DNA bands were visualized by the silver staining method. Developed gels were dried at room temperature before scoring the bands.

2.2.5 Band scoring and data analysis

Bands of different electrophoresis mobility were assumed to be non-allelic, while bands of the same mobility were assumed to be allelic. Only clear, unambiguous and reproducible bands were included in a datum matrix. Data were scored on the basis of the presence or absence of amplified fragment as a binary unit character (1 = presence, 0 = absence). Minor

Table 1 Released year, origin, and cotton-growing region of the tested cottons

Material	Released year	Origin	Cotton-growing region	Material	Released year	Origin	Cotton-growing region
Chuan 52-128	1952	Sichuan	YzRV	Lumian 9	1988	Shandong	YRV
Chuan 57-681	1957	Sichuan	YzRV	Lumian 11	1989	Shandong	YRV
CRI 3	1960	CAAS	YRV	Lu 1024	1986	Shandong	YRV
Zhong 8004	1968	CAAS	YRV	Sumian 2	1984	Jiangsu	YzRV
Zhong 8010	1968	CAAS	YRV	Sumian 3	1986	Jiangsu	YzRV
Liuzhuang 1	1966	Henan	YRV	Sumian 4	1987	Jiangsu	YzRV
Shannmian 4	1966	Shaanxi	YRV	Yanmian 48	1984	Jiangsu	YzRV
Shannmian 5	1969	Shaanxi	YRV	Simian 3	1989	Jiangsu	YzRV
Shannmian 9	1969	Shaanxi	YRV	Ekang 1	1988	Hubei	YzRV
Shann 401	1969	Shaanxi	YRV	Chuan 414	1984	Sichuan	YzRV
Kangbing dongtingmian	1966	Sichuan	YzRV	Chuanmian 109	1984	Sichuan	YzRV
Xiezuo 1	1966	Sichuan	YzRV	Mianwu 4176	1988	Sichuan	YzRV
Shannmian 6	1968	Shaanxi	YRV	Liaomian 10	1986	Liaoning	NVEM
CRI 9	1973	CAAS	YRV	Xinluzhong 3	1982	Xinjiang	NwIL
Zhong 3474	1975	CAAS	YRV	CRI 20	1994	CAAS	YRV
86-1	1976	CAAS	YRV	CRI 21	1994	CAAS	YRV
Yumian 1	1978	Henan	YRV	CRI 23	1995	CAAS	YRV
Jimian 1	1971	Hebei	YRV	Zhong 31	1998	CAAS	YRV
Jimian 3	1976	Hebei	YRV	Zhongzhi 86-6	1993	CAAS	YRV
Jizhi 17	1979	Hebei	YRV	Yumian 8	1992	Henan	YRV
Jinmian 8	1979	Shanxi	YRV	Yumian 19	1999	Henan	YRV
Shannmian 10	1971	Shaanxi	YRV	Yu 2067	1998	Henan	YRV
Shann 724	1974	Shaanxi	YRV	Yumian 11	1994	Henan	YRV
Shann 1155	1975	Shaanxi	YRV	Yumian 22	1999	Henan	YRV
Shann 3563	1975	Shaanxi	YRV	Yumian 10	1993	Henan	YRV
Shann 3619	1970	Shaanxi	YRV	Jimian 20	1990	Hebei	YRV
Shann 5245	1975	Shaanxi	YRV	Jizi 123	1998	Hebei	YRV
Jiangsumian 1	1970	Jiangsu	YzRV	Jimian 26	1998	Hebei	YRV
Chuan 73-27	1973	Sichuan	YzRV	Nongda 94-7	1998	Hebei	YRV
Qianjiang 9	1973	Zhejiang	YzRV	Lumian 12	1991	Shandong	YRV
Liaomian 7	1979	Liaoning	NVEM	Luwu 401	1991	Shandong	YRV
Guanglingmian	1973	Zhejiang	YzRV	Jinmian 14	1993	Shanxi	YRV
Mianxiang 1	1978	Henan	YRV	Jinmian 18	1995	Shanxi	YRV
CRI 12	1983	CAAS	YRV	Qinyuan 4	1998	Shanxi	YRV
CRI 15	1984	CAAS	YRV	Sumian 8	1995	Jiangsu	YzRV
CRI 19	1989	CAAS	YRV	Sumian 7	1993	Jiangsu	YzRV
Zhongkang 5	1980	CAAS	YRV	Sumian 11	1997	Jiangsu	YzRV
Zhong 206	1989	CAAS	YRV	Sumian 12	1997	Jiangsu	YzRV
Zhong 521	1982	CAAS	YRV	Ekang 3	1995	Hubei	YzRV
Zhong 6331	1986	CAAS	YRV	Xiangmian 16	1995	Jiangxi	YzRV
Yumian 4	1989	Henan	YRV	Chuanbei 2	1990	Sichuan	YzRV
Jimian 7	1980	Hebei	YRV	Chuan 243	1996	Sichuan	YzRV
Jimian 14	1983	Hebei	YRV	Liaomian 12	1994	Liaoning	NVEM
Jimian 15	1985	Hebei	YRV	Wanmian 11	1997	Anhui	YzRV
Jimian 19	1987	Hebei	YRV	Zhongzhi 372	1992	CAAS	YRV
Jihe 328	1981	Hebei	YRV	Siyang 168	1999	Jiangsu	YzRV
Jinmian 7	1983	Shanxi	YRV	Yuwu 1309	1991	Henan	YRV
Shann 8092	1981	Shaanxi	YRV				

polymorphic AFLP and SSR bands were excluded from the analysis because these can arise artifactually from differences in genomic DNA quality and other factors (Abdalla et al., 2001). Genetic similarity (GS) was estimated based on Nei and Li's coefficient (1979) using the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) Version 2.11a software package. Clustering analysis was performed using UPGMA (unweighted pair group method with arithmetic averages). The goodness of fit of the cultivars to a specific cluster in the UPGMA cluster analysis was determined by the Mental's correlation test (Mantel, 1967) of the NTSYS-pc (Version 2.11a) software. *PIC* for AFLP and SSR was calculated from the 1/0 datum matrix. *PIC* for SSR was calculated by $PIC = 1 - \sum p_i^2$, where p_i is the frequency of the allele at i for individual p (Ni et al., 2002; Warburton and Crossa, 2002), and *PIC* for AFLP was calculated with the following equation: $PIC = 1 - [f^2 + (1-f)^2]$, where f is the frequency of the marker in the AFLP data set (De Riek, 2001).

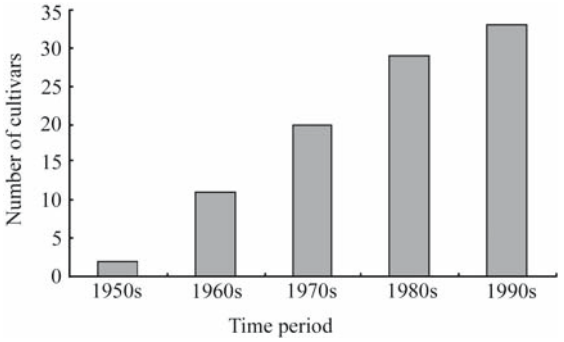

Fig. 1 The number of cotton cultivars of five different breeding periods

3 Results

3.1 Level of AFLP and SSR polymorphism

In the present study, 10 *Eco*RI and 10 *Mse*I primers constituted 100 primer combinations (10×10) for screening out polymorphic primers. Among them, 20 primer pairs produced good polymorphisms, clear and scorable band patterns. A total of 1 480 bands were generated from the 20 primer combinations among 95 genotypes, 214 of which (14.5%) were polymorphic. The number of bands and the degree of polymorphism revealed by each primer combination were given in Table 2. The number of total bands per primer pair ranged from 47 to 109, with an average of 74.0. The average value of polymorphic markers was 10.7 for each primer combination. The most efficient primer combination, with the largest number (33) of polymorphic bands, was E-ACA/M-CTA. But E-ACT/M-CAT produced the least number (1) of polymorphic bands. Molecular weight of the selected marker-bands approximately ranged from 50 bp to 600 bp.

Among the 40 SSR primers screened, 19 SSR primers generated consistently good amplification and polymorphism among the cotton cultivars. Eighty-nine DNA bands were obtained, 61 of which were polymorphic. The average polymorphism rate was 68.5%, much higher than that of AFLP (14.5%). The total number of alleles per locus ranged from 3 to 8, with an average of 4.7 per locus (Table 3). The maximum number of alleles was detected by BNL2634.

Table 2 AFLP amplifications of 20 primer combinations on 95 cottons and *PIC* values

Primer combination	Total number of bands	Number of polymorphic bands	Percentage of polymorphism /%	*PIC* value
E-AAC/M-CAC	96	5	5.2	0.04
E-AAC/M-CTG	74	6	8.1	0.06
E-AAC/M-TAT	109	12	11.0	0.11
E-AAG/M-CAC	94	3	3.2	0.03
E-AAG/M-CTA	88	12	13.6	0.10
E-AAG/M-CTG	65	3	4.6	0.04
E-AAG/M-TAT	78	3	3.9	0.03
E-ACA/M-CTA	107	33	30.8	0.24
E-ACC/M-CAT	65	6	9.2	0.05
E-ACC/M-CTA	63	16	25.4	0.13
E-ACG/M-CTA	47	13	27.7	0.12
E-ACT/M-CAT	69	1	1.4	0.02
E-AGG/M-CAT	70	18	25.7	0.15
E-AGG/M-CTA	77	24	31.2	0.20
E-AGG/M-CTG	51	17	33.3	0.16
E-AGG/M-TAT	78	8	10.3	0.07
E-AGC/M-CTA	62	8	12.9	0.07
E-AGC/M-CAA	72	16	22.2	0.13
E-AGC/M-CTT	62	4	6.5	0.03
E-GGA/M-CTA	53	6	11.3	0.05
Average	74.0	10.7	14.5	0.09

Table 3 Nineteen SSR primers used, map position, number of alleles of 95 cotton cultivars and their *PIC* values

SSR primer	Number of alleles	Number of polymorphic bands	Chromosome	*PIC* value
BNL530	6	4	4	0.79
BNL686	6	3	9sh	0.76
BNL840	5	5	26	0.53
BNL1053	5	4	3	0.79
BNL1317	4	4	9	0.66
BNL1414	6	3	9L	0.80
BNL1417	4	1	25	0.75
BNL1551	4	4	16	0.67
BNL1721	5	5	18L	0.71
BNL2448	4	3		0.60
BNL2634	8	4	16	0.85
BNL2960	4	4	10L	0.60
BNL3255	5	5	5S	0.62
BNL3408	4	3	3S\17L	0.56
BNL3449	3	1	16	0.67
BNL3479	4	1	18L	0.67
BNL3599	4	2	12S	0.67
BNL3816	3	1		0.67
CM43	5	4	20	0.77
Average	4.7	3.2		0.69

3.2 Diversity and the comparison of genetic similarity based on AFLPs and SSRs

Among all the tested accessions, the *PIC* values were different between the two marker systems (Table 2, Table 3). The *PIC* values for the 20 AFLP primer pairs varied from 0.01 (E-ACT/M-CAT) to 0.24 (E-ACA/M-CTA), with an average of 0.09. The *PIC* values of 19 SSR primer pairs ranged from 0.53 (BNL 840) to 0.85 (BNL 2634). The average *PIC* value for SSRs was 0.69, which was much higher than that of the AFLPs. Genetic similarity based on the AFLP and SSR markers showed some differences. The range of similarity coefficient of AFLPs was smaller than that of SSRs. And the average of similarity coefficient of AFLPs was larger than that of SSRs (Table 4).

3.3 Cluster analysis among the cultivars

The AFLP and SSR bands were used to determine the genetic distance among the entries. Genetic similarity estimates for the entire data set ranged from 0.978 to 0.998, and the average was 0.989 (Table 4). The highest genetic similarity was found

Table 4 Comparison of genetic similarity coefficients

Time periods	AFLP&SSR		AFLP		SSR		Number of cultivars	Major breeding method
	Average	Range	Average	Range	Average	Range		
1950s–1960s	0.989	0.982–0.994	0.992	0.987–0.997	0.915	0.833–0.976	13	Pedigree selection
1970s	0.989	0.981–0.996	0.993	0.984–0.998	0.908	0.843–0.975	20	Pedigree selection
1980s	0.989	0.978–0.995	0.993	0.988–0.997	0.914	0.776–0.983	29	Hybridization breeding
1990s	0.989	0.978–0.996	0.993	0.986–0.998	0.914	0.779–0.983	33	Hybridization breeding
Entire data	0.989	0.978–0.998	0.993	0.983–0.999	0.914	0.768–1.000	95	

between Shann 5245 and Chuanmian109 (0.998). Luwu 401 and Jinmian 14, Shann 401 and Jizhi 17 had the lowest genetic similarity (0.978). The high level of genetic similarity indicated that close genetic relationship and narrow genetic diversity existed among the tested cultivars. The Mental's test with correlation value of 0.840 indicated "good fit" for the cultivars to a specific cluster in the dendrogram. Utilizing similarity coefficient matrix based on the combined data from AFLP and SSR, a dendrogram was generated, showing the relationships among the accessions (Fig. 2). The clustering patterns could not be correlated to the geographic origin of the cultivars, since those from the same origin were found to be interspersed in different clusters. In addition, the clustering indicated no trend with respect to the pedigree and common parentage (pedigree data not shown). In fact, some of the cultivars derived from the same cross, such as Jimian 7 and Jimian 14 were obtained from (Ji 75-7 × Ji 75-23), did not grouped into the same subcluster.

In order to estimate the effects of intensive breeding over time, the complete data were assigned into different time periods as discussed in the section of Materials and methods. The summary of the similarity coefficient among the cultivars released during different time periods is presented in Table 4. The averages and ranges of genetic similarity from AFLP markers were very similar for all the time periods. However, ranges of genetic similarity based on SSR and AFLP&SSR markers showed some differences among different time periods. The ranges in the period from 1950s to 1970s were less narrowed than those from 1980s to 1990s.

4 Discussion

Analysis on genetic diversity of the genetic resources of disease-resistant cottons provides references not only for parental selection of breeding program but also for prediction of offspring variation, exploitation of heterosis and the construction of core collection. In the study, AFLP and SSR molecular markers were used for evaluation of genetic diversity among 95 China-bred cotton cultivars with Fusarium and/or Verticillium wilts resistance. Two hundred and fourteen polymorphic bands were produced with an average of 10.7 for each primer pair. Nineteen SSR primers generated 61 polymorphic bands. The average *PIC* values of AFLP and SSR were 0.09 and 0.69, respectively. Genetic similarity estimates for the entire data set ranged from 0.978 to 0.998 based on AFLP and SSR bands. At the molecular level, it had been proved that close genetic relationship and narrow genetic diversity existed within the tested cultivars.

As found in this study, the limited genetic diversity in cultivated upland cottons had ever been detected previously (Wendel et al., 1992; Brubaker and Wendel 1994; Tatineni et al., 1996; Zhang et al., 2005). The reasons why such a limited genetic diversity existed in the tested materials in this study may be ascribed to the emphasis on the application of several basic germplasms in the cotton improvement programs and rigorous selection for disease resistance. Tracing back to the breeding history of cotton in China, a few of American upland cottons such as Stoneville, Deltapine and King were introduced into China in the early 20th century, then extensively cultivated and utilized as parents in breeding program in China. Fusarium and Verticillium wilts were found in China in 1930s along with the introduction of the American upland cottons, and then spreaded rapidly and made serious damages. In order to minimize the losses that the two diseases caused, the Chinese breeders developed a number of resistant or tolerant cultivars through pedigree selection or hybridization by using the available genetic resources. The first Chinese Fusarium wilt resistant variety Chuan 52-128 was bred through pedigree selection from Delfose 531 cultivar in the severe disease nursery in 1952. Later, Chuan 57-681 and Chuan 57-50 with Fusarium wilt resistance were developed via pedigree selection from Deltapine 15 in 1957. In the same year, Liaomian 1 and Liaomian 2 with Verticillium wilt tolerance were bred through pedigree selection from the King cottons in the disease nursery. During 1960s and 1970s, 42 resistant cultivars had been bred through pedigree selection and hybridization using the resistant genetic resources in 1950s. From 1980s to 1990s, a hybridization method was extensively applied with a lot of cultivars developed through crosses of inter-cultivar previously bred. In addition, we hypothesize that vigorous selection for disease resistance is a potential mechanism for genetic bottlenecks. Much of the original genetic diversity of *G. hirsutum*, including some valuable alleles that confer to insects and environmental adversities resistance, maturity and fiber quality, would have been lost.

In the present study, an ambiguous classification was obtained within the tested cultivars based on AFLPs and SSRs. However, some of the tested cultivars showed obvious differences in morphological traits except for their resistance (data not shown). Combing use of these cultivars with those

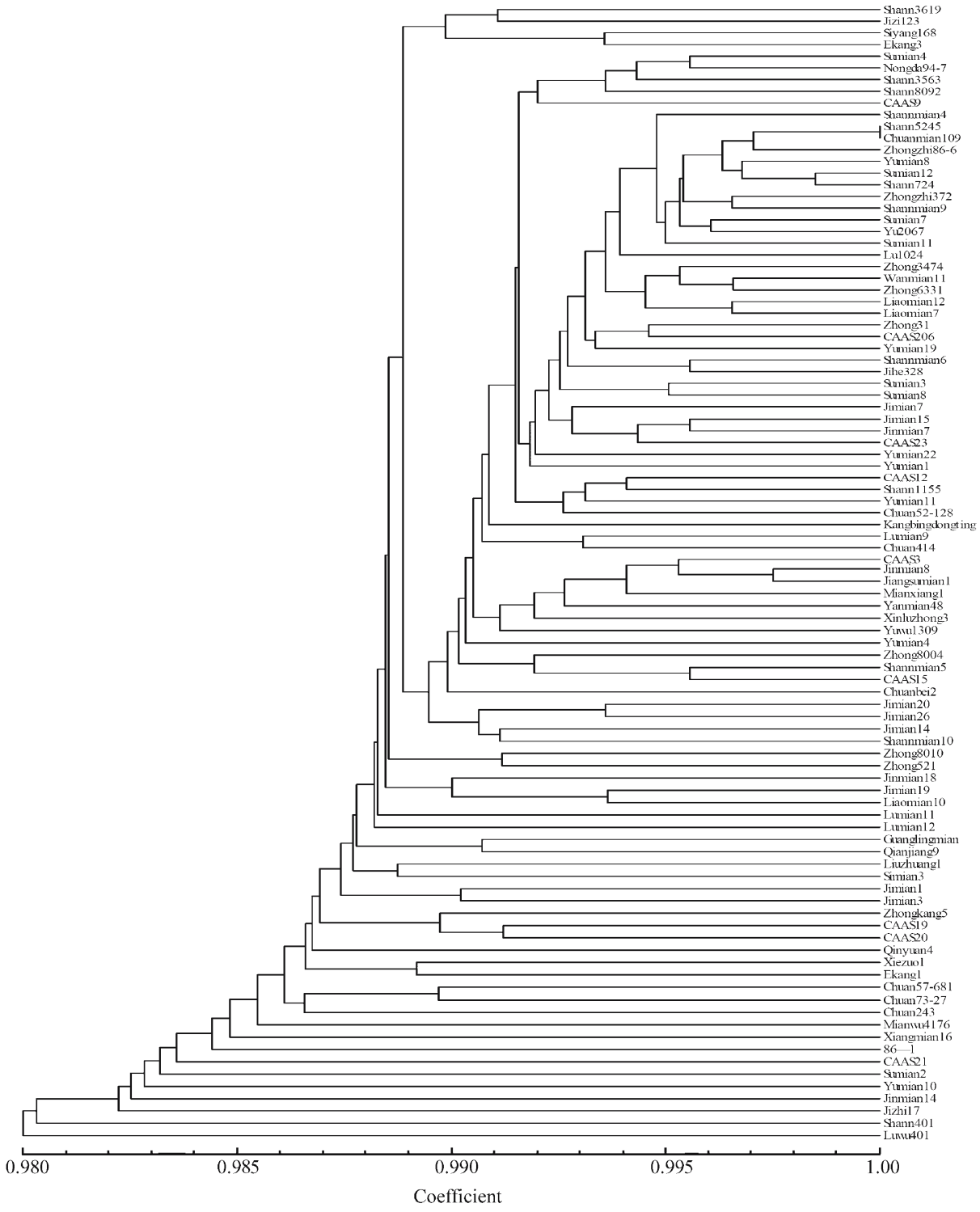

Fig. 2 A dendrogram of 95 cotton cultivars based on UPGMA cluster analysis and AFLPs and SSRs

of lower genetic similarity, such as between Luwu 401 and Jinmian 14, Shann 401 and Jizhi 17, can enhance our breeding efficiency in future cotton improvement program. Besides, the genetic base of the tested materials can be broadened by exploiting the diverse susceptible cultivars with other preferred agronomic traits. To reduce the genetic vulnerability of resistant *G. hirsutum*, other cultivated species such as *G. arboreum* and *G. barbadense* and some other wild cotton species can be utilized as genetic resources since they possess various superior traits, such as glandless seeds, fine fiber, high strength, the resistance of drought, alkalinity, diseases, insects and coldness.

Acknowledgements This research was supported by the National 973 Project (No. 2004CB117302), the Key Sci-Tech Project of the Ministry of Education of the People's Republic of China (No. 01010) and the Natural Science Foundation of Hebei Province (No. 301167, C2006001034).

References

Abdalla A M, Reddy O U K, El-Zik K M, Pepper A E (2001). Genetic diversity and relationships of diploid and tetraploid cottons revealed using AFLP. Theor Appl Genet, 102: 222–229

Brubaker C L, Wendel J F (1994). Reevaluating the origin of domesticated cotton (*Gossypium hirsutum*; Malvaceae) using nuclear restriction fragment length polymorphisms (RFLPs). Am J Bot, 81: 1309–1326

De Riek J, Calsyn E, Everaert I, Van Bockstaele E, De Loose M (2001). AFLP based alternatives for the assessment of distinctness uniformity and stability of sugar beet cultivars. Theor Appl Genet, 103: 1254–1265

Feng C D, Zhang J F, Liu J L, Sun J Z (1996). Analysis on the pedigree system of cotton cultivars (lines) with Fusarium wilt resistance. Acta Gossypii Sinica, 8(2): 65–70 (in Chinese)

Incirli A, Akkaya M S (2001). Assessment of genetic relationship in durum wheat cultivars using AFLP markers. Genet Resour and Crop Ev, 48(3): 233–238

Iqbal M J, Aziz N, Saeed N A, Zafar Y, Malik K A (1997). Genetic diversity evaluation of some elite cotton cultivars by RAPD analysis. Theor Appl Genet, 94(1): 139–144

Iqbal M J, Reddy O U K, El-Zik K M, Pepper A E (2001). A genetic bottleneck in the evolution under domestication of upland cotton *Gossypium hirsutum* L. examined using DNA fingerprinting. Theor Appl Genet, 103: 547–554

Kim M S, Moore P H, Zee F T, Fitch M M, Steiger D L, Manshardt R M, Paull R A, Drew R A, Ming R (2002). Genetic diversity of *Carica papaya* as revealed by AFLP markers. Genome, 45(3): 503–512

Liu S, Cantrell R G, McCarty J C Jr, Stewart J McD (2000). Simple sequence repeat-based assessment of genetic diversity in cotton race stock accessions. Crop Sci, 40: 1459–1469

Mantel N A (1967). The detection of disease clustering and a generalized regression approach. Cancer Res, 27: 209–220

Medini M, Hamza S, Rebai A, Baum M (2005). Analysis of genetic diversity in Tunisian durum wheat cultivars and related wild species by SSR and AFLP markers. Genet Resour and Crop Ev, 52: 21–31

Mellish A, Coulman B, Ferdinandez Y (2002). Genetic relationships among selected crested wheatgrass cultivars and species determined on the basis of AFLP markers. Crop Sci, 42: 1662–1668

Multani D S, Lyon B R (1995). Genetic fingerprinting of Australian cotton cultivars with RAPD markers. Genome, 38: 1005–1008

Nei M, Li W H (1979). Mathematical models for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA, 76: 5269–5273

Ni J J, Colowit P M, Mackill D J (2002). Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Sci, 42: 601–607

Paterson A, Brubaker C, Wendel J A (1993). A rapid method for extraction of *Gossypium* spp. genomic DNA suitable for RFLP or PCR analysis. Plant Biology Reporter, 11: 122–127

Rahman M, Hussain D, Zafar Y (2002). Estimation of divergence among elite cotton cultivars genotypes by DNA fingerprinting technology. Crop Sci, 42: 2137–2144

Rana M K, Bhat K V (2002). Genetic diversity analysis in Indian diploid cotton (*Gossypium* spp.) using RAPD markers. Indian J Genet, 62: 11–14

Rana M K, Singh V P, Bhat K V (2005). Assessment of genetic diversity in upland cotton (*Gossypium hirsutum* L.) breeding lines by using amplified fragment length polymorphism (AFLP) markers and morphological characteristics. Genet Resour and Crop Ev, 52: 989–997

Roberts C L, Staten G (1972). Heritability of Verticillium wilt tolerance in crosses of American upland cotton. Crop Sci, 12: 63–66

Satyavathi C T, Bhat K V, Bharadwaj C, Tiwari S P, Chaudhury V K (2006). AFLP analysis of genetic diversity in Indian soybean [*Glicine max* (L.) Merr.] cultivars. Genet Resour and Crop Ev, 53: 1069–1079

Small R L, Ryburn J A, Wendel J F (1999). Low levels of nucleotide diversity at homoeologous Adh loci in allotetraploid cotton (*Gossypium* L.). Mol Biol Evol, 16: 491–501

Soleimani V D, Baum B R, Johnson D A. (2002). AFLP and pedigree-based genetic diversity estimates in modern cultivars of durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.]. Theor Appl Genet, 104: 350–357

Tatineni V, Cantrell R G, Davis D D (1996). Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPDs. Crop Sci, 36: 186–192

Verhalen L M (1971). A quantitative genetic study of Verticillium wilt resistance among selected lines of upland cotton. Crop Sci, 11: 407–412

Vos P, Hoger R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res, 223: 4407–4414

Warburton M, Crossa José (2002). Data analysis in the CIMMYT applied biotechnology center for fingerprinting and genetic diversity studies. 2nd ed. Mexico D F: CIMMYT

Wendel J F, Brubaker C L, Percival A E (1992). Genetic diversity in *Gossypium hirsutum* and the origin of upland cotton. Am J Bot, 79: 1291–1310

Zhang J F, Lu Y, Cantrell R G, Hughs E (2005). Molecular marker diversity and field performance in commercial cotton cultivars evaluated in the southwestern USA. Crop Sci, 45: 1483–1490

Zhao H, Wang X F, Zhang G Y, Ma Z Y (2002). The study and establishment of AFLP technical system in cotton. J Agricultural University of Hebei, 25(1): 1–4 (in Chinese)