瑟伯氏棉和异常棉的陆地棉导入系的 EST-SSR 和 gSSR 分析

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摘要：用 EST-SSR 和 gSSR 分析了瑟伯氏棉和异常棉的 8 个导入系。结果表明，SSR 的扩增带可以分为两大类：第一大类是导入系和受体晋棉 6 号之间没有差异；第二大类是导入系和受体晋棉 6 号之间存在差异。第一大类可分为 5 个小亚类；第二大类中，对于 EST-SSR 来说，可分为 4 个小亚类：一是在晋棉 6 号中出现的条带在部分导入系中消失；二是条带大小与晋棉 6 号相比有变化；三是部分导入系中出现了瑟伯氏棉和异常棉的条带，但与晋棉 6 号相同的条带消失了；四是部分导入系中出现了供体和受体都没有的特异条带，而在另一些导入系中条带大小发生变化。对于 gSSR 来说，第二大类中又多了两个小亚类：即部分导入系中除了供体和晋棉 6 号都没有的条带；或供体和晋棉 6 号的条带同时出现在导入系中。这些供体和受体异常条带的增加和消失可能是 SSR 区域和 SSR 侧翼序列变化的结果。与 gSSR 相比，EST-SSR 在导入系和晋棉 6 号之间的变化更大，这可能是导致导入系比晋棉 6 号纤维品质更优的原因。

关键词：导入系；瑟伯氏棉；异常棉；EST-SSR；gSSR
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Characteristics of *Gossypium thurberi* and *G. anomalum* Introgression Lines of *G. hirsutum* Revealed by EST-SSR and gSSR

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Abstract: Eight introgression lines (ILs) of *G. thurberi* and *G. anomalum* were analyzed using EST-SSR and gSSR. The results showed that the bands detected by SSR markers could be sorted into two classes: (I) no differences between the eight ILs and Jinmian 6, the upland cotton receptor, and (II) differences among the eight ILs and Jinmian 6. In class I, five sub-classes can be identified. In class II, four sub-classes could be identified for EST-SSR: (1) bands detected in Jinmian 6 but absent in some ILs; (2) band size changed compared to Jinmian 6; (3) bands that introgressed from *G. thurberi* and *G. anomalum* while bands same as Jinmian 6 disappeared in some ILs; (4) the novel bands that differ from either the donor or the receptor appeared in some ILs, while bands size changed compared to Jinmian 6 in some other ILs. For gSSR, two additional sub-classes could be identified: (5) additional bands appeared in some ILs; (6) both donors' and Jinmian 6's bands appeared in ILs. The appearance of novel bands or disappearance of parental bands may result from the changes of SSR region and flanking sequence. Compared to the gSSRs, EST-SSRs were more variable between the ILs and Jinmian 6, which may be a result of the introgressed good fiber quality of the ILs.

Key words: introgression lines; *G. thurberi*; *G. anomalum*; EST-SSR; gSSR
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Cotton is the world's most important natural textile fiber, and it doubles as an oilseed crop. *Gossypium* contains about 50 diploid and tetraploid species distributed worldwidely in both tropical and subtropical areas. The diploid species (2n=2x=26) fall into eight different cytotypes designated as A, B, C, D, E, F, G, and K[1-2]. The tetraploid species (2n=4x=52, AADD) contain two distinct sub-genomes which are related to the A genome of the Asiatic cultivated diploid species and the D genome of the American wild diploid species[1].

Four *Gossypium* species, namely *G. arboreum*, *G. barbadense*, *G. herbaceum*, and *G. hirsutum* are cultivated. Upland cotton (*G. hirsutum* L.) dominates the cotton kingdom for its wide adaptation and high yield production. However, the fiber quality of upland cotton is not very good, and its improvement lag behind the development of textile industry.

The genetic variability for fiber traits is limited in the cultivated upland cotton germplasm. There is an urgent need to broaden the cotton gene pool by introgressing new genes from diverse sources to meet various challenges affecting fiber quality. Wild species display great variability, and they are potential sources of novel genetic variation for cotton improvement such as improving fiber quality, yield, stress tolerances, resistance to pest and disease, etc[3]. Some agronomically interesting traits, including low-gossypol seed and high-gossypol plants[4] and delayed pigment gland morphogenesis[5] have been transferred from wild species to cultivated upland cotton.

Microsatellites or simple sequence repeats (SSRs) are short tandemly repeated stretches of DNA composed of 1～6 bp. They have been shown to occur in both coding and non-coding regions of all higher organisms[6]. Some microsatellite sequences may be correlated with gene expression[7]; however, most of the microsatellite sequences are non-coding and their biological functions are not clear. Because of their abundance and high mutation rate, microsatellites have been extremely useful as genetic markers, especially for the purposes of investigating genetic variation and relatedness and performing linkage mapping[8-9].

G. thurberi (D_1) and *G. anomalum* (B_1) have genes for potential improvement of fiber quality[3]. In this study, enhanced fiber quality traits from these two diploid species were introduced into upland cotton, and the introgression lines (ILs) were identified by EST-SSR and gSSR.

1 Materials and Methods

1.1 Plant materials

Jinmian 6, an upland cotton cultivar of Shanxi Province in China, has high yield but poor fiber quality. In order to improve its quality, an introgression-breeding program was conducted. Two wild cottons, *G. thurberi* and *G. anomalum* were planted in greenhouse, and they were crossed to Jinmian 6. The crosses were made in 1994, and the F_1s were self-pollinated for four generations. During this procedure, fibers from each line were measured, and lines with good fiber quality were selected and backcrossed with Jinmian 6 for three generations. Finally, eight stable lines were generated. DH961-DH964 were from Jinmian 6×*G. thurberi*, and DH965-DH968 were from Jinmian 6×*G. anomalum* (Table 1). The eight ILs together with Jinmian 6 were introduced to our lab in 2003, and planted in 2004 on the farm of Huazhong Agricultural University.

Table 1 The fiber traits performance of materials used in this study

Traits	Jinmian 6	DH961	DH962	DH963	DH964	DH965	DH966	DH967	DH968
Fiber length/mm	29.1	32.6	32.7	33.1	33.9	33.1	33.4	33.3	32.9
Fiber strength/(cN·tex^{-1})	28.6	33.1	34.6	34.3	33.5	33.9	34.8	34.0	32.1
Micronaire	4.3	3.4	3.7	4.2	4.3	4.7	4.2	4.3	4.2

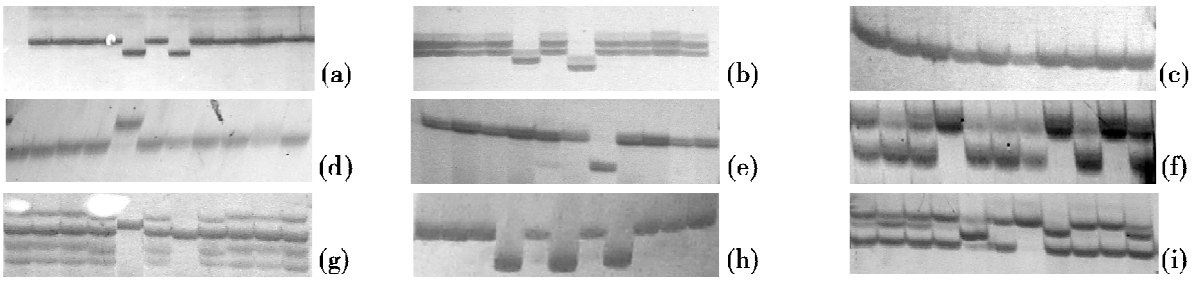
1.2 DNA marker analysis

Genomic DNA was isolated from frozen leaves of the eight ILs, Jinmian 6, *G. thurberi*, and *G. anomalum* using a CTAB procedure[10]. The MGHES-SSR primers derived from ESTs of upland cotton and CIR-SSR primers were used to analyze the eleven lines. The PCR reaction, electrophoresis, and silver staining were conducted as described by Lin et al[11].

2 Results

2.1 Characteristics of ILs detected by EST-SSR

The bands detected by MGHES primers can be sorted into two classes: (I) no differences between the eight ILs and Jinmian 6, and (II) differences between the eight ILs and Jinmian 6 (Fig. 1, Table 2). In class I, five sub-classes can be identified: (1) no differences between the eight ILs and Jinmian 6, and no differences between *G. thurberi* and *G. anomalum* (Fig. 1 a); (2) no differences between the eight ILs and Jinmian 6, and having differences between *G. thurberi* and *G. anomalum* (Fig. 1 b); (3) no differences between all eleven varieties (Fig. 1 c); (4) special for *G. thurberi* but no differences between the other varieties (Fig. 1 d); (5) special for *G. anomalum* but no differences between the other varieties (Fig. 1 e). In class II, four sub-classes can be identified: (1) bands that appeared in Jinmian 6 disappeared in some ILs (Fig. 1 f); (2) bands size changed compared to Jinmian 6 (Fig. 1 g); (3) bands that introgressed from *G. thurberi* and *G. anomalum* while bands same as Jinmian 6 disappeared in some ILs (Fig. 1 h); (4) the novel bands that differ from either the donor or the receptor appeared in some ILs, while bands size changed compared to Jinmian 6 in some other ILs (Fig. 1, i).



(a) I (1) produced by MGHES-52; (b) I (2) produced by MGHES-22; (c) I (3) produced by MGHES-10; (d) I (4) produced by MGHES-32; (e) I (5) produced by MGHES-31; (f) II (1) produced by MGHES-6; (g) II (2) produced by MGHES-16; (h) II (3) produced by MGHES-70; (i) II (4) produced by MGHES-75. From left to right: DH961, DH962, DH963, DH964, *G. thurberi*, Jinmian 6, *G. anomalum*, DH965, DH966, DH967 and DH968.

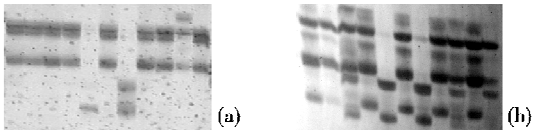
Fig. 1 Gel patterns revealed by MGHES markers

Table 2 Classification of gel patterns revealed by MGHES markers

Class	Sub-class	Markers	Percentage /%	Total /%
I	1	MGHES-14 MGHES-38a MGHES-43b MGHES-52 MGHES-59 MGHES-66	13.95	79.07
	2	MGHES-2 MGHES-10 MGHES-11a MGHES-15 MGHES-18 MGHES-21 MGHES-22 MGHES-27 MGHES-29 MGHES-30a MGHES-30b MGHES-37 MGHES-45 MGHES-46 MGHES-48 MGHES-57 MGHES-58	39.53	
	3	MGHES-33 MGHES-40 MGHES-50 MGHES-63 MGHES-71	11.63	
	4	MGHES-11b MGHES-12 MGHES-13 MGHES-32 MGHES-74	11.63	
	5	MGHES-31	2.33	
II	1	MGHES-6 MGHES-8 MGHES-60	6.98	20.93
	2	MGHES-16 MGHES-49	4.65	
	3	MGHES-70 MGHES-73	4.65	
	4	MGHES-44 MGHES-75	4.65	

2.2 Characteristics of ILs detected by gSSR

The bands detected by CIR primers also can be sorted into two classes: (I) no differences between the eight ILs and Jinmian 6, and (II) having differences between the eight ILs and Jinmian 6 (Fig. 2, Table 3). In class I, five sub-classes can also be identified as same as detected those by MGHES primers. In class II, two additional sub-classes can be identified: (5) additional bands appeared in some ILs; (6) both donors' and Jinmian 6's bands appeared in ILs (Fig. 2, Table 2).



(a) II (5) produced by CIR316; (b) II (6) produced by CIR156. From left to right: DH961, DH962, DH963, DH964, *G. thurberi*, Jinmian6, *G. anomalum*, DH965, DH966, DH967 and DH968.

Fig. 2 Additional gel patterns revealed by CIR markers

Table 3 Classification of gel patterns revealed by CIR markers

Class	Sub-class	Markers	Percentage /%	Total /%
I	1	CIR020 CIR048 CIR062 CIR094 CIR107 CIR121 CIR172 CIR222 CIR280 CIR354	10.10	88.89
	2	CIR009 CIR024 CIR032 CIR036 CIR038 CIR039 CIR040 CIR060 CIR069 CIR070 CIR078 CIR084 CIR086 CIR097 CIR100 CIR109 CIR119 CIR122 CIR143 CIR150 CIR158 CIR165 CIR166 CIR167 CIR168 CIR171 CIR175 CIR180 CIR187 CIR212 CIR224 CIR233 CIR234 CIR240 CIR254 CIR270 CIR272 CIR277 CIR278 CIR286 CIR289 CIR294 CIR305 CIR311 CIR338 CIR340 CIR347 CIR398 CIR408 CIR410 CIR411 CIR413	52.53	
	3	CIR015 CIR085 CIR139 CIR176 CIR219 CIR228 CIR295 CIR343	8.08	
	4	CIR047 CIR077 CIR105 CIR200 CIR210 CIR246 CIR261 CIR288 CIR292 CIR415	10.10	
	5	CIR063 CIR110 CIR112 CIR181 CIR283 CIR299 CIR344 CIR391	8.08	
II	1	CIR099	1.01	11.11
	2	CIR043 CIR068 CIR216 CIR381 CIR407	5.05	
	3	CIR183	1.01	
	4	CIR194	1.01	
	5	CIR013 CIR316	2.02	
	6	CIR156	1.01	

2.3 Introgression markers on single IL

Single IL harbored different numbers of introgression markers, ranging from 5 to 12. No single IL had all introgression markers. Among these introgression markers, MGHES8, MGHES73, and MGHES75 were more prone to be introduced than other EST-SSR markers; while CIR043, CIR099, and CIR407 were more prone to be introduced than other gSSR markers (Table 4).

Table 4 Introgression markers on single IL

ILs	Markers
DH961	MGHES-8, MGHES-49, MGHES-73, MGHES-75, CIR043, CIR099, CIR194, CIR407
DH962	MGHES-8, MGHES-73, MGHES-75, CIR013, CIR043, CIR099, CIR183, CIR194, CIR407
DH963	MGHES-73, MGHES-75, CIR013, CIR043, CIR099, CIR156, CIR194, CIR407
DH964	MGHES-6, MGHES-8, MGHES-16, MGHES-44, MGHES-49, MGHES-70, CIR043, CIR099,
DH965	MGHES-6, MGHES-8, MGHES-16, MGHES-44, MGHES-70, MGHES-75, CIR043, CIR099
DH966	MGHES-73, CIR013, CIR043, CIR156, CIR407
DH967	MGHES-6, MGHES-8, MGHES-44, MGHES-60, MGHES-73, CIR068, CIR099, CIR194, CIR216, CIR316, CIR381, CIR407
DH968	MGHES-73, MGHES-75, CIR013, CIR043, CIR156, CIR194

3 Discussion

In this study, the ILs of *G. thurberi* and *G. anomalum* were analyzed by EST-SSR and gSSR. It was obvious that most of the markers had no difference between the ILs and Jinmian 6. Compared to the gSSRs, EST-SSRs showed more differences between the ILs and Jinmian 6. As EST-SSRs were derived from expressed sequences, the more differences between the ILs and Jinmian 6 may be the results of their differences in fiber traits. The little difference between ILs may result from the different introgression markers that each IL harbored.

The SSR products on the gel in this study included two parts: the tandem repeat region and the conserved region surrounding the SSR. For some introgression markers, some bands were absent compared to Jinmian 6 and the donors, which indicted that the conserved region surrounding the SSR in ILs have changed. In addition, some new bands appearing in some ILs, which indicted that changes happened both in the tandem repeat region and in the conserved region surrounding the SSR, or base deletion, insertion and substitution happened in these regions. These phenomena also were found in analysis of allopolyploidization of new synthesized hexaploid wheat using SSR[12]. For those introgression markers, some markers were more prone to be introduced from *G. thurberi* and *G. anomalum* into upland cotton, which implied that some chromosome area existed in upland cotton which could be preferentially introgressed by other *Gossypium* species.

Microsatellites polymorphism associated with traits has been approved in plant. *Wx* gene on Chromosome 6 of rice encodes a kind of amylase, which is related to amylose in rice endosperm. The polymorphism of $(CT)_n$ in the 5′ UTR (untranslated region) of *Wx* gene is significantly related to the amylase content in rice[13-14]. In this study, more introgressions were observed in EST-SSRs, which may alter the expression of genes associated to fiber traits.

In upland cotton breeding, a genetic bottleneck was proposed[15]. In order to improve fiber quality and yield in upland cotton further, it is necessary to introduce genes that exist in other *Gossypium* species. *G. thurberi* and *G. anomalum* have potential genes to improve fiber quality[3]. In this study, *G. thurberi* and *G. anomalum* introgression lines of upland cotton with superior fiber quality were obtained and analyzed by EST-SSR and gSSR. The introgressed markers identified in this study would be helpful to introduce "positive" genes into upland cotton effectively.

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