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Molecular Markers of *Verticillium* Wilt Resistance in Upland Cotton (*Gossypium hirsutum* L.) Cultivar and Their Effect on Assisted Phenotypic Selection

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Abstract: Genetic population of F_2 and its corresponding $F_{2:3}$ lines derived from a hybridization combination of a *Verticillium* wilt disease resistance cultivar, of pest-resistant transgenic upland cotton (*Gossypium hirsutum* L.), crossed to a *Verticillium* wilt disease sensible upland cotton line (Luyuan 343), with high fiber quality genomic components introgressed from *G. barbadense* L., were used to evaluate the resistance of *Verticillium* wilt disease, and then dissected the resistance genes by SSR markers with the phenotype data of different developmental stage of cotton growth season. *qVWR*-16-1*a*, which was detected with the data of the vigorously developmental stage, is located between markers BNL2986 and NAU751 in the fragment of chromosome 16 with 5.73 cM to NAU751, and accounts for 16.53% of the phenotypic variation. The resistance is proved to be from the genotype of resistance parent. Meanwhile, with the data of the late stage of the cotton growth season, three QTLs were detected to be related to the *Verticillium* wilt resistance. *qVWR*-16-1*b* is located in the same interval to *qVWR*-16-1*a* with 1.73 cM to the marker NAU751 locus and accounts for 10.27% of the phenotypic variation, while another locus named *qVWR*-16-2*b* is located in another interval between BNL1604 and BNL1395, in the same chromosomal fragment with 1.39 cM to BNL1395, and accounts for 10.8% of the phenotype variation. Another QTL named *qVWR*-2-1*b* located in the interval between BNL3950 and BNL3971 with only 0.01 cM to BNL 3950 in the fragment of chromosome 2, accounts for 13.78% of the phenotypic variation. Evaluation on the disease resistance of some offspring (F_5) in the breeding population showed that pyramiding the resistant genotypes of marker NAU751 and BNL1395 can significantly improve the *Verticillium* wilt disease resistance. It was concluded molecular markers could be used to improve *Verticillium* wilt disease resistance in the process of breeding using the high fiber quality introgression line by assistant selection or pyramiding the resistant genotypes.

Key words: upland cotton (*Gossypium hirsutum* L.); *Verticillium* wilt disease resistance; SSR; QTLs; molecular marker assistant selection

1 Introduction

Gossypium hirsutum L. (commonly named upland cotton), is the most popular species and of prime economic importance in modern cotton production in the world due to its high lint yield. Improvements on fiber quality and disease resistance are the crucial aim of modern cotton breeding. In the process of cotton breeding, individu-

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als and lines with desirable cotton fiber quality such as fiber length, fiber strength and fiber fineness etc. can be easily screened by fiber quality test using specialized instruments. However, *Verticillium* wilt, caused by the soil-borne fungal pathogen *Verticillium dahliae* Kleb., is a major constraint to cotton production in nearly all the countries where cotton is cultivated. In China, the disease is present in all major cotton cultivated areas and causes significant loss of lint yield, and severe degression of fiber quality. Since cotton resistance to *Verticillium* wilt disease could only be well and truly evaluated under the stress conditions, there are practical constraints in performing resistance evaluations on large-scale breeding populations in disease garden with artificial-inoculation pathogens. So, it is not strange that some cotton varieties with high lint yield and relatively good fiber quality were usually sensible to *Verticillium* wilt disease. Inaccurate disease identification and consequently inefficient selection was one of the primary difficulties in synchronizing improvement on *Verticillium* wilt disease resistance and other agronomical traits in cotton breeding.

Characterization and mapping on the fundamental crop agronomy traits have been progressed and therefore molecular marker assistant selection (MAS) have been applied more and more in plant breeding. As the world's leading textile fiber crops, cotton (*Gossypium* spp.), especially the cultivated allotetraploid species (*G. hirsutum* L. and *G. barbadense* L.), benefited by the progress on molecular markers. Genetic maps of allotetraploid cotton species (*G. hirsutum* L. × *G. barbadense* L.) and upland cotton have been constructed based on kinds of molecular markers[1-5]. Genetic dissection by molecular markers on several cotton agronomical traits such as fiber quality[5-8], lint yield and its components[8-9], and maturity trait[10] etc. of cultivated upland cotton, and the attempts on marker assistant selection using markers related to fiber quality have been reported[11]. Mapping and molecular dissection on *G. barbadense* L. cotton *Verticillium* wilt disease resistance were conducted and markers related resistant genes or QTLs were identified using interspecific populations of *G. barbadense* L. × *G. hirsutum* L.[12-14]. However, since the segregation of interspecific population is severely deviant and the resistant phenotypes are hardly to be fixed, it is difficult to apply the markers for improving in the *Verticillium* wilt disease resistance. Wang et al. identified upland cotton *Verticillium* wilt disease resistance QTLs using SSR markers based on a population constructed by TM-1 crossed with a *Verticillium* wilt disease resistant cultivar[15]. But they did not consider applying the markers in practice of cotton breeding.

Here we report our molecular marker-facilitated work on genetic improving in the *Verticillium* wilt disease resistance of a disease sensible upland cotton introgression cultivar developed by researchers in China that have been used in cotton production and further development of high fiber quality upland cotton varieties due to the stable integration of the introgressed genomic composition related to high fiber quality from *G. barbadense* L. into the upland cotton genome[16].

2 Materials and Methods

2.1 Plant materials

Seeds of Luyuan 343 (LY343), a high fiber quality introgression upland cotton line sensible to cotton *Verticillium* wilt (The mean disease index was 53.9 in 2002 and 2003), were kindly supplied by the Institute of Application of Atomic Energy of Shandong Academy of Agricultural Sciences (IAAE, SAAS). Lumianyan 22 (LMY22) is an upland cotton cultivar with desirable resistance to cotton *Verticillium* wilt resistance (DI was authorized 18.3, and confirmed with a little fluctuation in our experiment) developed by our research group. All plant materials were self pollinated and field trials were carried out in the Experimental Station of Shandong Cotton Research Center (ES, SCRC) in Linqing

County, Shandong Province, China.

2.2 Population development and phenotype evaluation

The hybridization of LMY22 LY343 was performed in ES, SCRC in 2002, and F_1 plants were planted in Hainan Province in winter season of the same year. 265 individuals of F_2 population were planted in routine cotton trial field in ES, SCRC in 2003. Lines of derived $F_{2:3}$ populations, together with two parents, were planted in the disease garden with artificial inoculation of mixed *Verticillium* dahliae Kleb. isolated from typical cotton cultivated regions in Shandong Province. *Verticillium* wilt disease resistance was evaluated during the disease fastigium in 2004 in ES, SCRC by the following formula[17].

$$DI = \sum(N_i \times i) / \sum N_i \times i_{Max}$$

Where, DI, disease index; N, line number; i=0, 1,…4, disease degree from 0 to 4; i_{Max}, the maximum disease degree in the line evaluated.

2.3 DNA extraction, molecular markers and mapping

DNA samples of all parents and individuals of F_2 mapping population were isolated and purified followed the method described by reference[18] with slightly modified based on our previous report[19]. All primers used in this study were chosen from http://www.cottonssr.org and the EST-SSR (NAU) developed by Nanjing Agricultural University[20] and synthesized by Invitrogen Co. Ltd in China (www.invitrogen.com.cn). The PCR reaction for SSR and surveyed by PAGE/silver staining was performed followed our previously reported method[21]. All trait means and correlations were calculated by using the SAS program. The linkage map was made using Mapmaker/Exp (Version 3.0) and the QTL likelihood map, gene action, and phenotypic variance (PV) explained by individual QTLs were determined by interval mapping using WinQTLCart2.5[22].

And the polymorphic loci not in the linkage group were analyzed by ANOVA with the segregation data of the marker genotype and the *Verticillium* wilt disease index to see if they are statically significantly related to the *Verticillium* wilt resistance.

2.4 Improvements of *Verticillium* wilt resistance in progenies of breeding population

Two generations of continuous individual selection (one in ES, SCRC and another in Hainan Province) every year, aimed at integrative agronomical traits, especially the higher fiber quality, were conducted from F_3 progenies of the population using conventional breeding methods in routine cotton trial field. The F_5 offsprings were used to evaluate the effects of identified *Verticillium* wilt disease resistance related markers in disease garden of ES, SCRC, by statistical analysis, on the mean differences of the disease resistance of progenies with different genotypes of markers or marker combinations.

3 Results

3.1 Segregation of *Verticillium* wilt resistance in F_2 and $F_{2:3}$ population

The F_2 individuals and $F_{2:3}$ lines showed phenotypic distribution of the *Verticillium* wilt resistance, indicating by ranging disease degree and disease index (DI) (Fig. 1, 2 and 3). Figure 1 showed continuous distribution of disease degree with highest frequency of degree 1. Figure 2 represented the DI distribution in July when the *Verticillium* wilt disease was not very slightly happened (the mean DI was 28.28) while Figure 3 represented the distribution in August when the disease was seriously happened (the mean DI was 51.64). The population was distributed normally for the trait as a whole, but the mean was shifted a little toward the sensible parent (LY343). It indicated that the *Verticillium* wilt disease resistance in this population was controlled by quantitive trait loci. The transgressive segregation of *Verticillium* wilt disease resistance in the population was observed slightly but evidently (Fig. 3). This indicated that progenies better than the resistant parent in re-

sistant trait could be expected if we used the population for breeding.

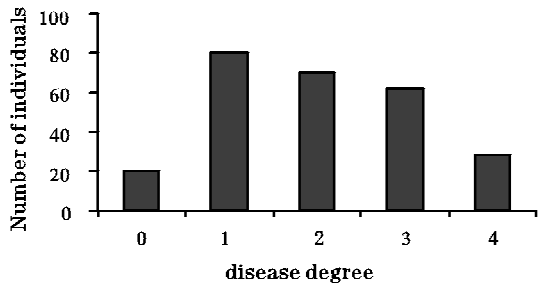
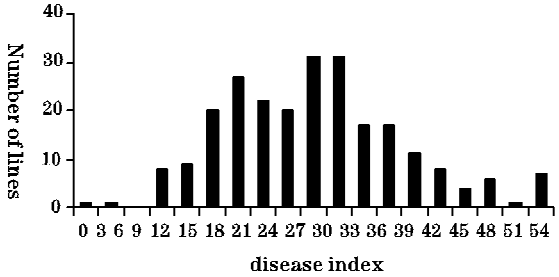
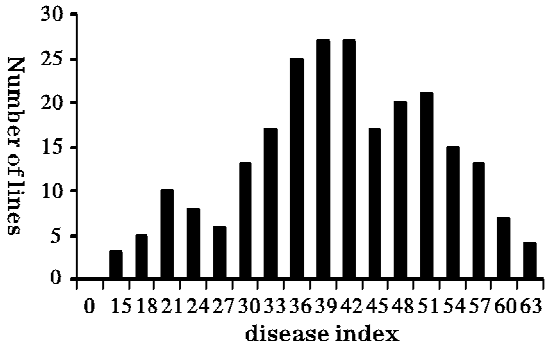


Fig. 1 Evaluation of resistance to *Verticillium* wilt disease in F_2 population by cotton stem dissection



The data were evaluated in July and the DI value of the parents was not shown in this figure.

Fig. 2 Frequency distribution of the individual $F_{2:3}$ lines for disease index of *Verticillium* wilt



Mean disease index of the two parents, LMY22 and LY343, are at the range pointed by the arrows.

Fig. 3 Frequency distribution of the individual $F_{2:3}$ lines for disease index of *Verticillium* wilt

3.2 Polymorphic SSR loci analysis

Among the total 317 SSR primer pairs (217 BNL and 100 NAU) used in this study, 24 primer pairs (23 BNL and 1 NAU) involved individual loci respectively can be detected with polymorphism between the parents. Higher ratio (10.6%) of polymorphic BNL SSR primers was detected than that of NAU primers (only 1%). It is possible that we should select SSR primers in the bulky Cotton DB based on the genetic background of materials used and the primary aim of the experiment carried out.

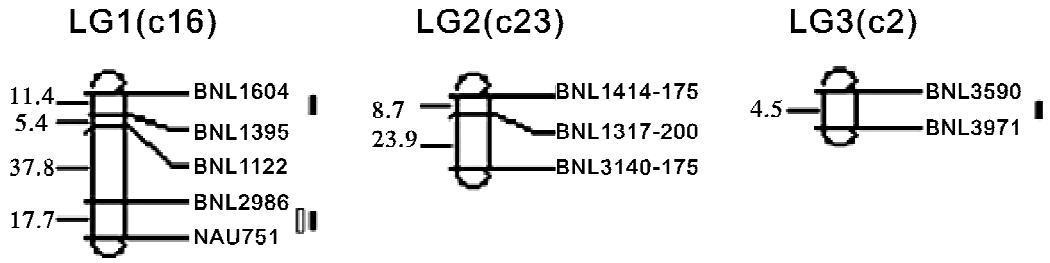
3.3 SSR loci associated with *Verticillium* wilt resistance

All polymorphic marker loci were performed linkage analysis using MAPMAKER/EXP (version 3.0) with linkage criteria of LOD 3 and a recombination frequency of 0.5. Among the seven marker loci related to *Verticillium* wilt disease, BNL1395, NAU751 and BNL3590, BNL3971 were found located in two linkage groups (LG1 and LG3) respectively (Figure 4), while BNL827, BNL3474 and BNL830 could not link to any linkage groups. The two linkage groups were most possibly located on chromosome 16 (C16) and chromosome 2 (C2) referring to the Cotton Microsatellite DataBase (http://www.cottonssr.org) and the published allotetraploid cotton linkage map[2].

Only one QTL (*qVWR*-16-1*a*) was determined by interval mapping using WinQTLCart 2.5[22] to be related to the *Verticillium* wilt resistance using the data collected in July (Figure 4). It is located in the interval between the markers BNL2986 and NAU751 in the fragment of C16 with 5.73 cM to NAU751 and accounts for 10.27% of phenotypic variation (Table 1). Three QTLs were determined to be related to the *Verticillium* wilt resistance using the data collected in August (Figure 4). *qVWR*-16-1*b* is located in the interval between markers BNL2986 and NAU751 in the fragment of C16 with 1.73 cM to NAU751 and accounts for 10.27% of phenotypic variation, while another locus named *qVWR*-16-2b is located in another interval between BNL1604 and BNL1395 in the same chromosomal fragment with 1.39 cM to BNL1395 and accounts for 10.8% of phenotypic variation. Another QTL named *qVWR*-2-1b is located in the interval between BNL3590 and BNL3971 with only 0.01 cM to BNL 3590 in the fragment of C2 , and accounts for 13.78% of

phenotypic variation. It is notable that additive effects are the main genetic effects of the QTLs except *qVWR*-2-1b with very large dominant effects besides lower LOD value (Table 1).

The markers BNL1395and BNL3590 were proved to be statistically significantly related to *Verticillium* wilt resistance ($P<0.05$). However, the LOD values of *qVWR*-16-2b and *qVWR*-2-1b closely linked to the two markers were relatively small.



□ indicates the QTL detected in the vigorously developmental stage (July) while ■ denotes QTLs detected in late stage of the cotton growth season (Aug.). LG1-LG3 is the names of linkage groups while C16, C23 and C2 are the chromosomes where the linkage groups located. The unit of the distance between the loci is centi-Morgan (cM).

Fig. 4 *Verticillium* wilt resistance QTLs detected in different cotton developmental stage

For marker loci that not linked to any other locus, only BNL3279 was statistically significantly related to *Verticillium* wilt resistance ($P<0.05$) when analyzed with the data collected in July. BNL827 and BNL830 was proved to be statistically significantly related to *Verticillium* wilt resistance ($P<0.05$) when analyzed with the data collected in August. We did not take this marker into consideration for its genetic effect on the disease resistance in further marker effects evaluation.

Table 1 QTLs for *Verticillium* wilt resistance in a $F_{2,3}$ population from Lumianyan 22×Luyuan 343

	QTLs	Chr.	Interval	Nearest marker	LOD	a	d	Var/%
July	*qVWR*-16-1*a*	Chr. 16	BNL2986-NAU751	NAU751	3.5986	-5.3222	2.2194	16.53
August	*qVWR*-16-1*b*	Chr. 16	BNL2986-NAU751	NAU751	2.0693	-3.4550	0.0442	10.27
	qVWR-16-2*b*	Chr. 16	BNL1604-BNL1395	BNL1395	1.7324	3.3799	-0.6236	10.80
	qVWR-2-1*b*	Chr. 2	BNL3590-BNL3971	BNL3590	1.2593	-4.2853	2.7965	13.78

3.4 Effects evaluation on selection by SSR loci associated with *Verticillium* wilt resistance

The *Verticillium* wilt resistance related SSR markers detected by interval mapping mentioned above were applied to 138 F_5 lines derived from continuous selection on fiber quality, which were planted in disease garden at ES, SCRC in 2006 to evaluate disease resistance of every individual line. Statistical analysis on marker genotype/disease phenotype correlations is presented in Table 2.

The differences of mean DI between the homozygous genotypes of marker BNL1395 and NAU751 are 11.344 ($P=0.0116$) and 6.434 ($P=0.0184$) respectively and with statistical significance. The results indicated that the effect would be evident if we selected the individuals with genotype of resistance marker either of BNL1395 or NAU751. Since the mean DI difference between homozygote pyramiding both resistance genotypes of BNL1395 or NAU751 and homozygote with both non-resistance genotypes is 18.383 with extremely statistical significance ($P=0.0001$), selection on the recombinants with the two resistance marker genotypes would be more effective on improvement of *Verticillium* wilt disease in our breeding population. The difference of mean DI between the homozygous genotypes of marker BNL3590 is 5.721 but with no statistical significance, this marker seemed to

be of little value in MAS.

4 Discussions

4.1 Molecular dissection and genetic improvement on cotton *Verticillium* wilt resistance

Precise selection on individuals well and truly resistant to *Verticillium* wilt is one of the main constraints in cotton *Verticillium* wilt disease breeding, since it is unpractical to conduct all breeding populations under strict selection on the artificial inoculated disease garden. And even in disease garden, the uniformity and stability of disease occurrence are difficult to control. The progress on molecular markers in genetic dissection on important agronomical traits provided ways and means to efficient evaluation and selection on cotton *Verticillium* wilt disease resistance. However, only a few work about molecular marker research on genes or QTLs related to cotton *Verticillium* wilt disease resistance using populations of either interspecific crosses by *G. hirsutum* L. × *G. barbadense* L.[12-14] or intraspecific crosses[15] with no further considerations of breeding application.

Table 2 The genotype of SSR marker linking with *Verticillium* wilt resistance and the disease index in F_3 breeding population

Markers	Genotype	No. of lines	Mean DI	Difference	*F* value	*P* value	Direction
BNL1395	A_1A_1	33	32.935				LY343
	a_1a_1	78	44.280	11.344	7.091	0.0116*	
	A_1A_1	33	32.935				
	A_1a_1	17	38.317	5.382	2.882	0.1068	
	A_1a_1	17	38.317				
	a_1a_1	78	44.280	5.963	3.208	0.0877	
NAU751	A_2A_2	68	35.402				LMY22
	a_2a_2	10	41.836	6.434	5.985	0.0184*	
	A_2A_2	68	35.402				
	A_2a_2	21	37.943	3.541	0.992	0.3316	
	A_2a_2	21	37.943				
	a_2a_2	40	41.836	2.893	0.457	0.5140	
BNL3590	A_3A_3	66	37.763				LMY22
	a_3a_3	36	43.484	5.721	1.753	0.1949	
BNL1395+NAU751	A_1A_1 A_2A_2	12	28.985				
	a_1a_1 a_2a_2	27	47.368	18.383	21.044	0.0001**	
	A_1A_1 A_2A_2	12	28.985				
	A_1A_1	33	32.935	3.951	2.443	0.129	
	A_1A_1 A_2A_2	12	28.985				
	A_2A_2	68	35.402	6.417	7.745	0.0071**	

Note: * Significance at $P<0.05$, ** Significance at $P<0.01$.

The occurrence of *Verticillium* wilt disease was closely related with the cotton growth and development. In the process of *Verticillium* wilt disease resistance breeding, vigorous and serotinous individuals are frequently noticed to be "resistance", however, this kind of individuals may not possess the genetic basis of resistance especially the so called "horizontal resistance"[23]. The resistance phenotype is in fact something like "avoidance". Therefore, it is most important to choose actual resistance materials and accurate resistance evaluation methods. In this present study, resistance QTLs detected in different developmental stage with the same population was somewhat different. Among the three QTLs detected with the data of the late stage of cotton growth season, only one QTL (*qVWR*-16-1b) was similar to the QTL (*qVWR*-16-1a) detected with the data of the vigorously developmental stage. The other two QTLs (*qVWR*-16-2b and *qVWR*-2-1b) could not be detected in the

vigorously developmental stage (Table1). It is possible that the vigorously plant growth covers up the disease occurrence and therefore disturbs the QTL detection. In conclusion, for mapping the cotton *Verticillium* wilt disease resistance by molecular markers, it is necessary to use the data collected from the late stage of cotton growth season when the *Verticillium* wilt disease was seriously occurred.

4.2 Potentials of using molecular marker assistant selection on *Verticillium* wilt resistance

Individual lines of F_5 selected by resistance phenotype either homozygotes of BNL1395 or NAU751, the SSR markers tightly linked to loci of *Verticillium* wilt resistance detected in this study, were proved in possession of cotton *Verticillium* wilt resistance while the sole resistance homozygote phenotype of BNL3590 was proved of little use to improve the resistance because of its insignificantly statistical correlation with *Verticillium* wilt resistance (Table 2). Since the independent QTL confers only a part and limited contribution to their corresponding phenotype, pyramiding different QTLs guided by molecular markers should get more ideal phenotype. Here, as in Table 2, if individuals harbored both homozygotes of the resistance genotype of BNL1395 and NAU751 were selected, statistically significant disease resistance improvement would be foreseeable in later breeding populations. Because BNL1395 and NAU751 were located in the same linkage group with distance and their resistance genotypes came from different parents, the number of recombinant genotypes ($A_1 A_1 A_2 A_2$ and $a_1 a_1 a_2 a_2$) was expected similarly less within progenies in breeding population with no purposeful selection on *Verticillium* wilt disease. However, it noted that the number of lines with marker genotype $A_1 A_1 A_2 A_2$ was obviously less compared with the number of lines with marker genotype $a_1 a_1 a_2 a_2$ (Table 2).

Considering the fact that not all progenies harbored one or both homozygous resistance alleles are with desirable disease resistance in this paper, precise mapping to *Verticillium* wilt disease resistance using as more as possible markers should be conducted since it is possible that more alleles and interactions among all alleles involved in the disease resistance in cotton. Further work of genetic dissection on other agronomical traits and their interactions by molecular markers is now in processing in our lab and will be reported later.

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References:

[1] REINISCH A J, Dong J M, Brubaker C L, et al. A detailed RFLP map of cotton, *Gossypium hirsutum* × *Gossypium barbadense*: chromosome organization and evolution in disomic polyploid genome [J]. Genetics, 1994, 138:829-847.

[2] ZHANG J, Guo W, Zhang T. Molecular linkage map of allotetraploid cotton (*Gossypium hirsutum* L. × *Gossypium barbadense* L.) with a haploid population [J]. Theor Appl Genet, 2002, 105:1166-1174.

[3] BLENDA A, Scheffler J, Scheffler B, et al. A cotton microsatellite database resource for *Gossypium* genomics[J]. BMC Genomics, 2006, 7:132.

[4] WAGHMARE V N, Rong J, Rogers C J, et al. Genetic mapping of a cross between *Gossypium hirsutum* (cotton) and the Hawaiian endemic, *Gossypium* tomentosum[J]. Theor Appl Genet, 2005, 111:665-676.

[5] LUO Ming, Li Ming-yang, Hou Lei, et al. An AFLP marker related to fibrogenesis in upland cotton (*Gossypium hirsuturm* L.) [J]. Journal of Ge-

netics and Genomics, 2001, 28:677-682.

[6] YUAN You-lu, Zhang Tian-zhen, et al. Molecular tagging and mapping of QTLs for super quality fiber properties in upland cotton [J]. Journal of Genetics and Genomics, 2001, 28:1151-1161.

[7] ZHANG T, Yuan Y, Yu J, et al. Molecular tagging of a major QTL for fiber strength in Upland cotton and its marker-assisted selection [J]. Theor Appl Genet, 2003, 106:262.-268.

[8] WU Mao-qing, Zhang Xian-long, Ni Yi-chun, et al. localization of QTLs for yield and fiber quality traits of tetraploid cotton cultivar [J]. Journal of Genetics and Genomics, 2003, 30: 443-452.

[9] WU Yao-ting, Zhang Tian-zhen, Yin Jian-mei. Genetic diversity detected by DNA markers and phenotypes in upland cotton [J]. Journal of Genetics and Genomics, 2001, 28:1040-1050.

[10] FAN Shu-li, Yu Shu-xun, Song Mei-zhen, et al. Construction of Molecular Linkage Map and QTL Mapping for Earliness in Short-season Cotton [J]. Cotton Science, 2006, 18: 135-139.

[11] GUO Wang-zhen, Zhang Tian-zhen, Ding Ye-zhang, et al. Molecular marker assisted selection and pyramiding of two QTLs for fiber strength in upland cotton [J]. Journal of Genetics and Genomics, 2005, 32:1275-1285.

[12] Gao Yu-qian, Nie Yi-chun, Zhang Xian-long. QTL mapping of genes resistant to *Verticillium* wilt in cotton [J]. Cotton Science, 2003, 15:73-78.

[13] DU Wei-shi, Du Xiong-ming, Ma Zhi-ying. Studies on SSR markers of resistance gene of *Verticillium* wilt in cotton [J]. Jour of Northwest Sci-Tech Univ of Agri and For (Nat Sci Ed), 2004, 32:20-24.

[14] QI Jun-sheng, Ma Cun, Zhang Yuan-en, et al. AFLP markers on *Verticillium* wilt resistance gene of Island cotton (*Gossypium barbadense* L.) [J]. *Acta Phytopathologica Sinica*, 2001, 31: 63-68.

[15] WANG Hong-mei, Zhang Xian-long, He Dao-hua, et al. Detection of DNA markers associated with resistance to *Verticillium dahliae* in cotton [J]. *Acta Phytopathologica Sinica*, 2005, 35:333-339.

[16] SU Xue-he, Gao Guo-qiang, Shi Xiang-yu, et al. Breeding of a terrestrial long-staple cotton—Luyuan 343 [J]. *Acta Agriculturae Nucleatae Sinica*, 2000, 14:180-183.

[17] SHEN Qi-yi. The disease of cotton-basic research and control [M]. Beijing: Scientific Press, 1992: 264-269.

[18] PATERSON A H, Brubaker C L, Wendel J F. A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis [J]. Plant Molecular Biology Reporter, 1993, 11(3):122-127.

[19] WANG Fu-rong, Zhang Jun, Liu Ren-zhong, et al. Genetic analysis of upland cotton germplasm obtained from introduction DNA of island cotton [J]. Chinese Agricultural Sciences, 2005, 38: 1528-1533.

[20] HAN Z G, Wang C B, Song X L, et al. Characteristics, development and mapping of *Gossypium hirsutum* derived EST-SSRs in allotetraploid cotton [J]. Theor Appl Genet, 2006, 112:430-439.

[21] ZHANG Jun, Wu Yao-ting, Guo Wang-zhen, et al. Fast screening of microsatellite markers in cotton with PAGE silver staining [J]. *Acta Gossypii Sinica*, 2000, 12:267-269.

[22] ZENG Z B, Kao C H, Basten C J. Estimating the genetic architecture of quantitative traits [J]. Genet Res, 1999, 74:279-289.

[23] FLOR H H. Current status of the gene-for-gene concept [J]. Annul Review of Phytopathology, 1971, 9:275-296.