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# Fine mapping of the dominant glandless Gene $Gl_2^e$ in Sea-island cotton (*Gossypium barbadense* L.)

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**$Gl_2^e$ is a mutant gene that controls glandless trait in cotton plants and seeds. It is an important gene resource to gossypol-free cottonseed breeding. The objective of this research was to develop SSR markers tightly linked with $Gl_2^e$ by using the $F_2$ segregating population containing 1599 plants derived from the cross of *G. hirsutum* genetic standard line TM-1 and *G. barbadense* glandless mutant line Hai-1. Genetic analysis suggested that the $Gl_2^e$ was an incomplete dominant gene. Based on the backbone of genetic linkage map from *G. hirsutum* × *G. barbadense* $BC_1$ published by our laboratory, $Gl_2^e$ was located between CIR362 and NAU2251b, NAU3860b, STV033, with a genetic distance 9.27 and 0.96 cM, respectively. This result is useful for cloning $Gl_2^e$ gene by map-based cloning method.**

cotton, $Gl_2^e$ gene, glandless frait, molecular marker, fine mapping

Cotton (*Gossypium* spp.) is one of the most important economic crops in China. It is not only the leading natural fiber resource, but also an excellent source of oil and protein. Dark-colored glands are a special organ in all *Gossypium* plants[1]. These glands contain high levels of gossypol and related terpenoids, which are toxic to human beings and monogastric animals[2]. So a large number of nutrient-rich resources cannot be sufficiently applied because of the presence of toxic gossypol within seed glands; however, other plant tissues such as shoot and root containing toxic gossypol in nature can increase the resistance of cotton to pests and pathogens. Therefore, gossypol and gland have long been a hot topic. Besides, how to develop glandless cotton varieties, especially those with low gossypol such as the glanded-cotton with glandless-cottonseeds, has been a more important problem confronting cotton breeders[1].

Afifi *et al.*[3] found a glandless mutant in Sea-island cotton (*G. barbadence* L.) by $^{32}P$ irradiation, named 'Bahtim 110'. Genetic analysis demonstrated that this character was controlled by a single dominant gene, named $Gl_2^e$, which could efficiently inhibit the formation of cotton gland[4]. Cotton Research Institute (CRI) of Chinese Academy of Agricultural Sciences (CAAS) handed out the *G. barbadense* glandless mutant Hai-1 in 1986. According to genetic analysis, Tang et al.[5] also showed that glandless trait in Hai-1 was controlled by $Gl_2^e$. This provided a new germplasm for low gossypol cotton breeding. Compared with glandless line controlled by duplicate recessive genes $gl_2gl_3$, natural crossing did not happen easily and resulted in immingled variety for Hai-1 line controlled by $Gl_2^e$. Additionally, $Gl_2^e$ gene could be transferred into the excellent upland cotton background by backcross and dramatically accelerate breeding efficiency[5]. The glandless trait could also serve as an excellent marker character for cotton heterosis utilization[6].

Classical genetic analysis suggested that $Gl_2$ and $Gl_3$ were located respectively on the chromosomes 12 and 26

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in cotton[7]; therefore $Gl_2^e$ as its multiple alleles is also located on the chromosome 12. Yu et al.[8] identified an SSR marker with a 1.9 cM genetic distance of $Gl_2^e$ gene by Bulk-segregant analysis (BSA) using $F_2$ population derived from the cross of *G. hirsutum* genetic standard line TM-1 and the isogenic line ESP. Recently, development of the cotton genomic research and saturation of genetic linkage map facilitate the gene fine mapping. In this study, we constructed an $F_2$ segregating population derived from the cross of *G. hirsutum* genetic standard line TM-1 and *G. barbadense* glandless mutant line Hai-1. By analyzing genetic linkage map from *G. hirsutum* × *G. barbadense* $BC_1$ published by our laboratory[9], we found linked markers closely linked with gene $Gl_2^e$ by molecular markers technique. The objectives were to provide a foundation for map-based cloning and further cultivation of the glanded-cotton plants with glandless cottonseeds varieties via gene engineering.

## 1 Materials and methods

### 1.1 Plant materials

The upland cotton genetic standard stock, "Texas Marker-1" (TM-1) was kindly made available from the Southern Plains Agricultural Research Center, USDA-ARS[10]. Hai-1, a *G. barbadense* glandless mutant, was provided by CRI, CAAS in 1986[5]. All materials were stored by self-crossing for several years in our lab.

TM-1 was crossed with Hai-1 in Jiangpu Experimental Station, Nanjing Agricultural University. The $F_1$ hybrid plants were self-pollinated to produce $F_2$ seeds in winter at Hainan Province in 2005. 1599 $F_2$ individuals derived from a single $F_1$ hybrid were planted in Nanjing in 2006. The gland traits of different positions of plants were investigated, including cotyledon, hypocotyls, the first leaf, stripe, bract, bud and calyx in the seedling stage, bud stage and florescence.

### 1.2 DNA extraction

Genomic DNAs from 1599 $F_2$ individuals, $F_1$ and two parents were extracted as described by Paterson et al.[11].

### 1.3 SSR analysis

SSR primers were separately obtained from the following sources: BNL primers from Research Genetics Co. (Huntsville, AL, USA, http://www.resgen.com); JESPR from Reddy et al.[12]; CIR from Nguyen et al.[13]; STV from Scheffler et al.[14]; NAU developed from EST-SSR in our lab[15,16]. All primers information could be downloaded from http://www.genome.clemson.edu/projects/cotton. To name the markers, the letter in each marker describes the origin of marker, followed by the primer number, and "a" or "b" describes the polymorphism locations of marker in two parents.

Taq polymerase, dNTPs and other reagents came from Tiangen Biotech (Beijing) Co., Ltd. Polymerase chain reaction (PCR) product electrophoresis and protocol for SSR analysis were the same as reported by Zhang et al.[17].

### 1.4 Data analysis

Chi-square test was used to check if the normal segregation occurred by SPSS13.0 software[18]. Joinmap 3.0[19] was employed to construct the linkage map (LOD>6.0, recombination fraction 50 cM). The Kosambi mapping function was used to convert recombination frequencies into map distances (centiMorgans, cM).

## 2 Results and analysis

### 2.1 Genetic analysis of glands traits

A large quantity of glands emerged in main organs of TM-1 plants; almost no glands could be observed in Hai-1 plants; and a few glands distributed in $F_1$ plants. To determine the genetic characters of $Gl_2^e$ gene, we investigated the different positions and stages of 1599 $F_2$ segregating plants. The results showed that 441 plants had dark-colored glands, 761 plants with low-glands, and 427 glandless plants respectively (Figure 1). The data by $\chi^2$ were consistent with a segregation ratio 1∶2∶1 ($\chi^2 = 4.028 < \chi^2_{0.05,2} = 5.99$), which further confirmed that glandless character was controlled by an incomplete dominance gene, $Gl_2^e$.

### 2.2 Fine mapping of $Gl_2^e$

SSR markers each with a 5-cM interval were selected on the genetic linkage map of chromosome 12 in our lab (Figure 2(a)) to screen the parent plants. The markers with the polymorphism were used to amplify the DNA of 210 $F_2$ segregating plants selected randomly and the marker genotypes were recorded. After eliminating the segregation distortion markers by $\chi^2$ test, other SSR markers were detected by linkage analysis. The results showed that $Gl_2^e$ was located between CIR362 and NAU5079. Further analysis was performed using the markers between CIR362 and NAU5079. Ultimately,

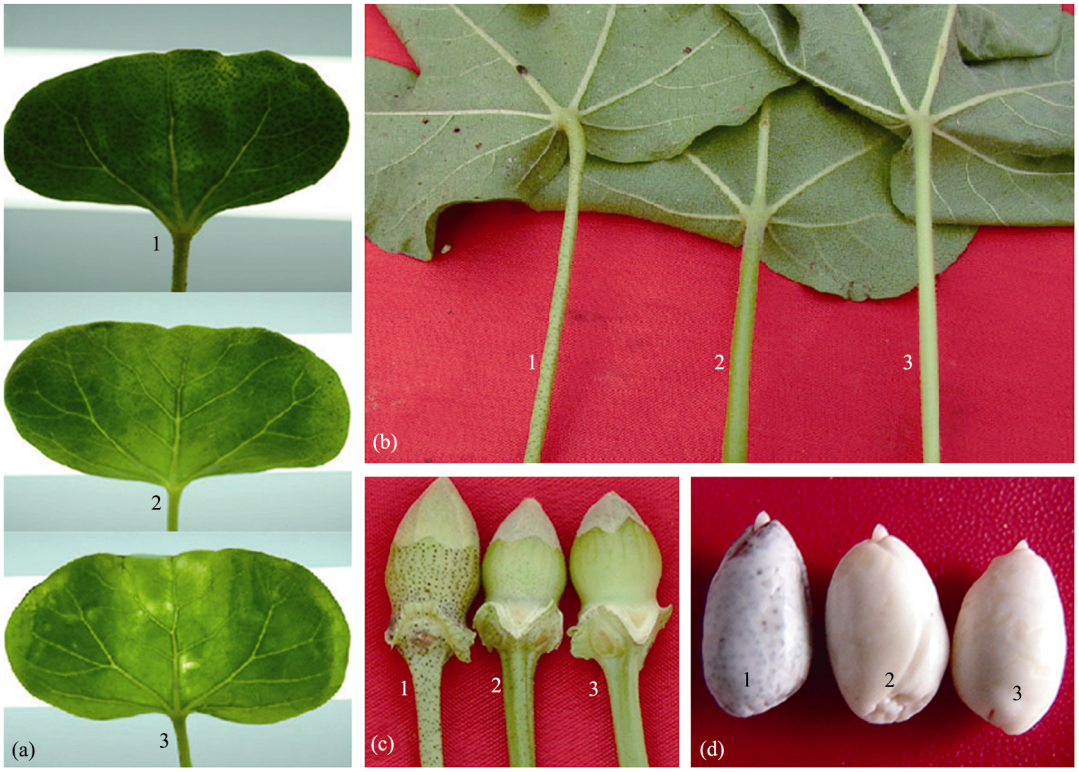


**Figure 1** Phenotypes of the mutated glandless gene $Gl_2^e$ investigated in the different tissues. (a)—(d) indicate cotyledon, stripe, bud and seed, respectively. 1—3 indicate TM-1(gland), $F_1$ (low-gland) and Hai 1(glandless), respectively.

$Gl_2^e$ was located between CIR362 and NAU2251b, NAU3860b, STV033 with genetic distance 11.03 and 1.19 cM, respectively (Figure 2(b)).

According to the preliminary results above, 1599 plants of the $F_2$ segregating population were further screened using the markers highly linked with $Gl_2^e$. Four markers of the above together with 3 markers linked with $Gl_2^e$ on both sides of the interval were selected. The results showed that NAU3778 and CIR362 were located on the same side of $Gl_2^e$ with genetic distance 11.25 and 9.27 cM respectively; NAU2251b (NAU3860b, STV033 with the same position), NAU445 and CIR302 were located on the other side with genetic distance 0.96, 5.29 and 7.11 cM, respectively (Figure 2(c)). Amplified polymorphic bands of primer NAU2251 are given in Figure 3.

## 3 Discussion

*G. bickii*, as well as other Australian diploid wild species, has a special trait of delayed pigment gland morphogenesis[20]. It is glandless in the seeds but glanded in all other plant parts including cotyledon, hypocotyls and other organs and tissues after seedling emerged. By the distant hybridization techniques, Zhu et al.[21] had obtained successfully a fertile upland cotton germplasm that possessed characteristics of delayed pigment gland morphogenesis. However, the upland cotton cultivars have never been developed. At present, researchers have cloned a number of genes related to gossypol biosynthesis[22−28] and further provided useful information for the mechanism of gossypol biosynthesis. Recently, Sunilkumar et al.[29] have successfully used RNAi in combination with seed special promoter to disrupt gossypol biosynthesis in cottonseed tissue by interfering with the expression of the $\delta$-cadinene synthase gene (CAD1) during seed development. As a result, cottonseed gossypol levels were reduced in a stable and heritable manner and the plant-gossypol levels were not diminished. This result laid a foundation for controlling gene expression artificially by molecular techniques and for the regulation of gossypol synthesis. In this study, $Gl_2^e$ is a qualatative trait gene and is not impacted by environment. Map-based cloning of the gene and study of gossypol regulation mechanism are of great theoretical and

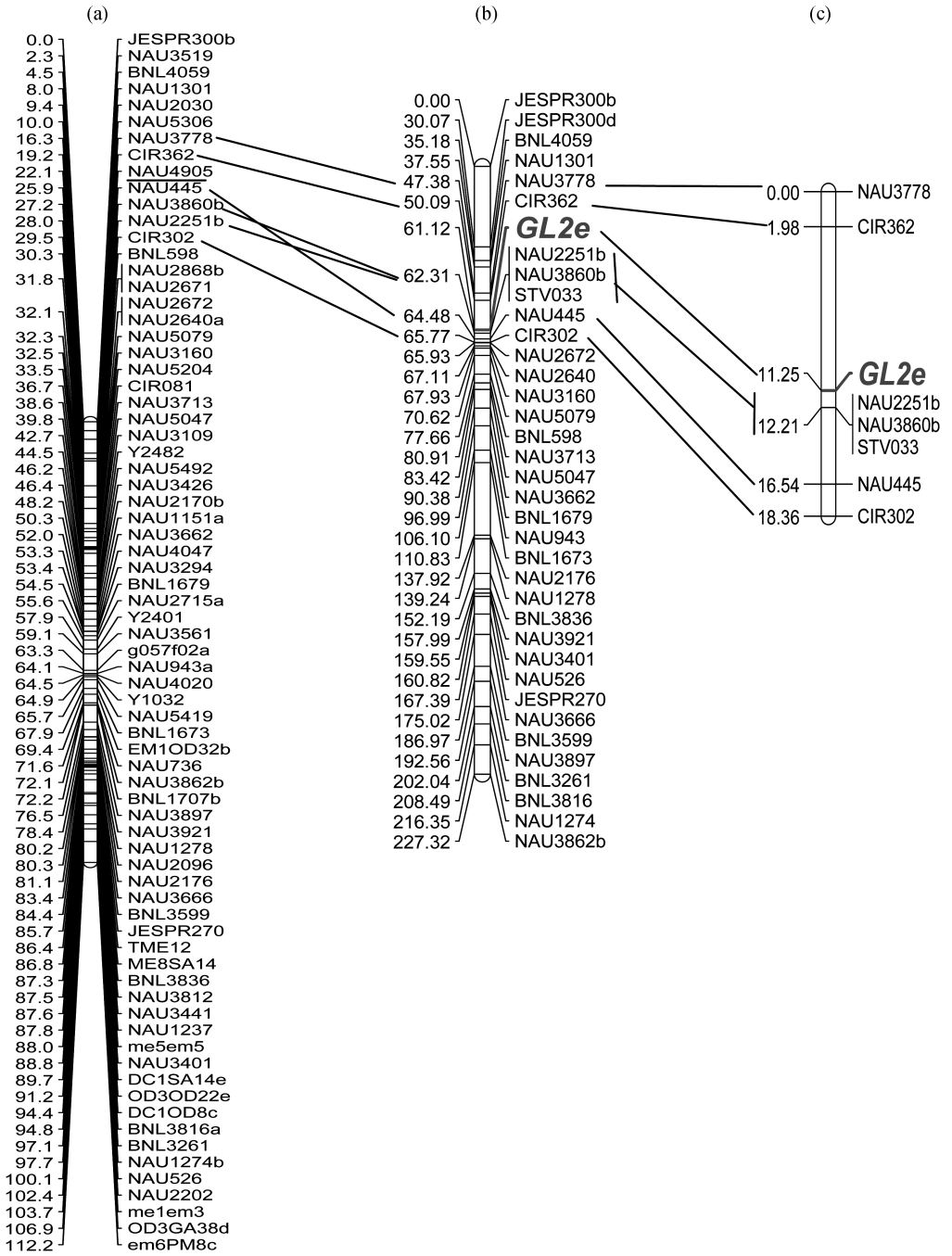


**Figure 2** Genetic linkage map of $Gl_2^e$. (a) The genetic linkage map of chromosome 12 from *G. hirsutum* × *G. barbadense* $BC_1$ published by our laboratory, the underlined marker indicates segregation distortion marker; (b) mapping of $Gl_2^e$ in chromosome 12; (c) fine mapping of $Gl_2^e$ in chromosome 12.

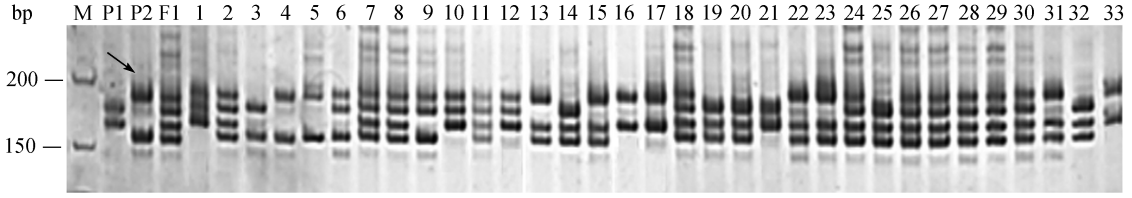


**Figure 3** Amplified polymorphic bands of primer NAU2251 in the $F_2$ population of TM-1 with Hai1. Arrowhead indicates polymorphic bands of NAU2251b tagged in chromosome 12. M, Marker; $P_1$, TM-1; $P_2$, Hai-1; $F_1$, (TM-1×Hai-1)$F_1$; others lanes (1－33) are $F_2$ individuals.

practical significance, not only in clarifying the mechanism of gossypol biosynthesis in depth, but also in breeding cotton varieties of the glanded-plants with glandless cottonseeds by genetic engineering.

The objective of gene mapping is to tag the interest gene in a very small region. In this case, it is possible to predict physical distance by genetic distance if the mapping population is large enough. Preliminary mapping cannot precisely measure the genetic distance between gene and marker due to the small mapping population. Genetically, the larger the mapping population is, the smaller candidate region of interest gene is[30]. In this study, 210 $F_2$ segregating plants were selected randomly and $Gl_2^e$ gene was located in an interval of 12.22 cM. Further, $Gl_2^e$ gene was located in the interval of 10.23 cM after 1599 plants were examined. The result dramatically increased the accuracy of the location and provided a reliable guarantee for further map-based cloning of the gene.

Yu et al.[8] identified an SSR marker with 1.9 cM genetic distance apart from $Gl_2^e$ gene. And physical analysis of "ESP" Bacterial Artificial chromosome (BAC) libraries has yielded contigs consisting of putatively positive BAC clones. However, this BAC library was not applied widely due to long genetic distance and small mapping population. In this study, NAU2251b, NAU3860b and STV033 with 0.96 cM genetic distance screened can be regarded as a probe to screen positive clones from BAC library and new and more closely linked markers can be exploited by BAC positive clones terminal sequencing. The study laid a good foundation for further cloning of the gene. At present, further research on this gene is in progress.


1 Cotton Research Institute, Chinese Academy of Agricultural Sciences. Genetics and Breeding of Cotton in China. Shandong: Shandong Science & Technology Press, 2003. 562
2 Bell A A, Stipanovic R D. The chemical composition biological activity and genetics of pigment glands in cotton. Proc Beltwide Cotton Prod Res Conf, 1977. 244－258
3 Afifi A, Bary A A, Kamel S A, et al. Bahtim 110, a new strain of Egyptian cotton free from gossypol. Emp Cot Grow Rew, 1966, 43: 112－120
4 Kohel R J, Lee J A. Genetic analysis of Egyptian glandless seeds cotton. Crop Sci, 1984, 24: 1119－1121
5 Tang C M, Min L F, Zhang T Z, et al. Genetic analysis for hai 1 strain of glandless cotton (*G. barbadence* L.) interaction between $Gl_2^e$ and $Gl_1$. Cotton Sci Sin, 1996, 8: 138－140
6 Yuan Y L, Jing S R, Zhang T Z, et al. Studies on dominant glandless marker trait applied to heterosis in upland cotton. Seed, 1997, 6: 15－17
7 Lee J A. The genomic allocation of the principal foliar-gland loci in *Gossypium hirsutum* and *Gossypium barbadense*. Evolution, 1965, 19: 182－188
8 Yu J, Kohel R J, Dong J M, et al. Toward positional cloning of a major glandless gene in cotton. Proc Beltwide Cotton Conf, National Cotton Council, Memphis, TN, 2000. 516－517
9 Guo W Z, Cai C P, Wang C B, et al. A microsatellite-based, gene-rich linkage map reveals genome structure, function, and evolution in *Gossypium*. Genetics, 2007, 176: 527－541
10 Kohel R J, Lewis C F, Richmond T R. Texas marker-1: Description of a genetic standard for *Gossypium hirsutum* L. Crop Sci, 1970, 10: 670－671
11 Paterson A H, Brubaker C L, Wendel J F. A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP and PCR analysis. Plant Mol Biol Rep, 1993, 11: 122－127
12 Reddy O U K, Pepper A E, Saha S. New dinucleotide and trinucleotide microsatellite marker resources for cotton genome research. J Cotton Sci, 2001, 5: 103－113
13 Nguyen T B, Giband M, Brottier P. Wide coverage of the tetraploid cotton genome using newly developed microsatellite markers. Theor Appl Genet, 2004, 109: 167－175
14 Taliercio E, Allen R D, Essenberg M, et al. Analysis of ESTs from multiple *Gossypium hirsutum* tissues and identification of SSRs. Genome, 2006, 49: 306－319
15 Han Z G, Guo W Z, Song X L, et al. Genetic mapping of EST-derived microsatellites from the diploid *Gossypium arboreum* in allotetraploid cotton. Mol Gen Genomics, 2004, 272: 308－327
16 Han Z G, Wang C B, Song X L, et al. Characteristics, development and mapping of *Gossypium hirsutum* derived EST-SSRs in allotetraploid cotton. Theor Appl Genet, 2006, 112: 430－439
17 Zhang J, Wu Y T, Guo W Z, et al. Fast screening of microsatellite markers in cotton with PAGE/silver staining. Cotton Sci Sin, 2000, 12: 267－269
18 Li Z H, Luo P. SPSS for Windows. 2rd ed. Beijing: Publishing House of Electronics Industry, 2005. 87－89
19 van Ooijen J W, Voorrips R E. JoinMap Version 3.0: Software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen. 2001
20 Fryxell P A. A revision of Australian species of *Gossypium* with observation on occurrence of Thespesia in Australia (Malvaceae). Austr J Bot, 1965, 18: 71－102
21 Zhu S J, Jiang Y R, Reddy N, et al. Breeding, introgression and inheritance of delayed gland morphogenesis trait from *Gosspium bickii* into upland cotton germplasm. Chin Sci Bull, 2004, 49: 2470－2476
22 Chen X Y, Chen Y, Heinstein P, et al. Cloning, expression and characterization of (+)-δ-cadinene synthase: A catalyst for cotton phytoalexin biosynthesis. Arch Biochem Biophys, 1995, 324: 255－266
23 Liu C J, Heinstein P, Chen X Y. Expression pattern of genes encoding farnesyl diphosphate synthase and sesquiterpcne cyclase in cotton suspension cultured cells treated with fungal elicitors. Mol Plant-Microbe Inter, 1999, 12: 1095－1104
24 Meng Y L, Jia J W, Liu C J, et al. Coordinated accumulation of (+)-8-cadinene synthase mRNAs and gossypol in developing seeds of *Gossypium hirsurum* and a new member of the cad1 family from *G. arboreum*. J Nat Prod, 1999, 62: 248－252
25 Tan X P, Liang W Q, Liu C J, et al. Expression pattern of (+)-δ-cadinene synthase genes and biosyn thesis of sesquiterpcne aldehydes in plants of *Gossypium arboreum* L. Planta, 2000, 210: 644－651
26 Luo P,Wang Y H, Wang G D, et al. Molecular cloning and functional identification of (+)-δ-cadinene-8-hydroxylase, a cytochrome P450 momooxygenase(CYP70-6B1) of cotton sesquiterpene biosynthesis. Plant J, 2001, 28: 95－104
27 Xu Y H, Wang J W, Wang S, et al. Characterization of GaWRKY1, a cotton transcription factor that regulates the sesquiterpene synthase gene (+)-δ-cadinene synthase-A1. Plant Physiol, 2004, 135(1): 507－515
28 Chang P A, Li B, Ni X M, et al. Molecular cloning and expression analysis of a RanBP2 zinc finger protein gene in upland cotton (*Gossypium hirsutum* L.). Colloids Surf B Biointerfaces, 2007, 55: 153－158
29 Sunilkumar G, Campbell L M, Puckhaber L, et al. Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. Proc Natl Acad Sci USA, 2006, 103: 18054－18059
30 Li W T, Zeng R Z, Zhang Z M, et al. Fine mapping of locus *S-b* for $F_1$ pollen sterility in rice (*Oryza sativa* L.). Chin Sci Bull, 2006, 51: 675－680