Chinese Science Bulletin 2004 Vol. 49 No. 23 2470—2476

Breeding, introgression and inheritance of delayed gland morphogenesis trait from *Gosspium bickii* into upland cotton germplasm

ZHU Shuijin, JIANG Yurong, Reddy Naganagouda & JI Daofan

College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310029, China
Correspondence should be addressed to Zhu Shuijin (e-mail: shjzhu@zju.edu.cn)

Abstract **A tri-specific hybrid with delayed pigment gland morphogenesis was obtained by crossing the amphidiploid of (*G. arboreum*×*G. bickii*) F_1 and an upland cotton germplasm with pigment gland genotype of $Gl_2Gl_2gl_3gl_3$. The tri-specific hybrid was a typical interspecific hybrid with high sterile, and the chromosome configuration at meiosis MI of PMC was $2n = 52 = 41.04\,\mathrm{I} + 4.54\,\mathrm{II} + 0.57\,\mathrm{III} + 0.04$. The crossover value of bivalent was 1.19. Two fertile plants with objective character were obtained in BC_8 population by continuously backcrossing with $Gl_2Gl_2gl_3gl_3$ as recurrent parent to the tri-specific hybrid, and a new upland cotton germplasm, named ABH-0318, with delayed pigment gland morphogenesis trait was developed through selfing and screening. The pigment gland trait of ABH-0318 was stable, and there were almost no pigment glands observed in the dormant seeds, although there were a few pigment glands confined to cotyledon edges, and the gossypol content in the dormant seeds was 0.017% only, being a typical low gossypol cotton type. However, a large quantity of pigment glands emerged in cotyledons and other main organs of plant after seed germination, and the gossypol contents in the upper parts of the plant were similar to that of ordinary glanded cotton types. Genetic analysis demonstrated that the delayed pigment gland morphogenesis trait of this germplasm was controlled by the interaction of the genes located in two pigment gland loci, Gl_2 and Gl_3. Among them, the gene located in locus of Gl_2, derived from *G.bickii*, was dominance to upland cotton pigment gland alleles, Gl_2 and gl_2, but was recessive epistatic to another glanded gene Gl_3, which was named Gl_2^b temporarily. While the gene located in the locus of Gl_3 was a recessive gene come from upland cotton.**

Keywords: *G. bickii*, *G. arboreum*, *G. hirsutum*, pigment gland, gossypol, interspecific hybrid.

DOI: 10.1360/982004-255

Cotton is a valuable economic crop. The cottonseed after ginning is made up of fuzz, kernel and hull, among which kernel constitutes about 50% to the seed weight. Cottonseed kernel of *G. hirsutum* contains about 40% of protein and more than 35% of cottonseed oil, which obviously is a potential rich source of high quality protein and edible oil supplement[1,2]. However, the utilization of cottonseed is limited by the presence of gossypol and its derivatives in seeds of ordinary glanded cotton cultivars, which are toxic to human and non-ruminant animals[3]. McMichae[4—7] discovered the glandless mutant in upland cotton germplasm, *G.hirsutum* var. *Punctatum* Hopi M, and developed further a glandless cotton cultivar controlled by double recessive genes, named '23B', in which the whole plant including seeds was free from gossypol glands. Since then, a new era of utilization of protein products from cottonseed had been initiated. Afifi et al.[8] developed another glandless germplasm for cotton breeding of *G. barbadense*, named 'Batim110', by the method of irradiation mutant breeding, in which the whole plant glandless trait was controlled by a single dominant gene. However, as the glandless plants are completely free from gossypol glands that act as a protective factor, the normal glandless cotton cultivars are more susceptible to many diseases and phytophageous insects[9—11], which led to the greatly limitation in production.

G. bickii Prokh ($2n$ = 26, genome G), as well as other Australian diploid wild species in genome C, has a special trait of delayed pigment gland morphogenesis, namely, it is glandless in the seeds but glanded in all other plant parts including cotyledon, hypocotyls and other organs and tissues after seedling emerged[12]. If this unique characteristic can be transferred into cultivated upland cotton, undoubtedly, the ideal cotton would possess virtues of both glanded and glandless cotton and accordingly improves its economic properties by producing glandless and low gossypol content seeds for more extensive uses without any loss in production due to the pest incidence because of the high level of gossypol glands in the plant parts except in seeds. Zhang et al.[13] obtained an amphidiploid of (*G. arboreum*×*G. bickii*) F_1 with the special trait of delayed pigment gland morphogenesis by method of interspecific hybridization and chromosome duplication. We then used this amphidiploid as donor parent to cross and backcross with an upland cotton germplasm with pigment gland genotype of $Gl_2Gl_2gl_3gl_3$. A special fertile upland cotton germplasm, named 'ABH-0318', with delayed pigment gland morphogenesis trait was obtained in the BC_8 population. In this paper, we report the breeding program employed to develop this germplasm and genetic analysis of the delayed pigment gland morphogenesis trait, which would provide a basis for breeding upland cotton cultivars with low gossypol seeds only in the future.

1 Materials and methods

(i) Crossing parents. The amphidiploid of (*G. arboreum*×*G. bickii*) F_1 ($2n$ = 52, genome AAGG) with the glandless seed and glanded plant trait was used as a female parent, which was obtained by Shanxi Agricultural University through the interspecific hybridization and

Table 1 Cotton materials used in the experiment and their main characteristics

Materials	Gland genotype	Main characteristics	Sources
G. bickii	Unknown	Wild cotton species, glandless seeds and glanded plant	Cotton Research Institute, CAAS
AAGG[a)]	Unknown	Amphidiploid, glandless seeds and glanded plant	Shanxi Agricultural University
TM-1	$Gl_2Gl_2Gl_3Gl_3$	Upland cotton standard line, glanded plant and seeds	Cotton Research Institute, CAAS
ZMS_{13}	$gl_2gl_2gl_3gl_3$	Glandless cotton cultivar, glandless seeds and plant	Zhejiang University
$Gl_2Gl_2gl_3gl_3$	$Gl_2Gl_2gl_3gl_3$	Gland gene marker line, glanded seeds and plant	Cotton Research Institute, CAAS
$gl_2gl_2Gl_3Gl_3$	$gl_2gl_2Gl_3Gl_3$	Gland gene marker line, glanded seeds and plant	Cotton Research Institute, CAAS

a) AAGG as the amphidiploid of (*G. arboreum*×*G. bickii*) F_1, the same below.

chromosome doubling. Upland cotton germplasm with pigment gland genotype of $Gl_2Gl_2gl_3gl_3$ was taken as male parent in crossing and recurrent backcrossing. Additionally, cultivars such as ‘ZMS_{13}’ (glandless cotton cultivar with pigment gland genotype of $gl_2gl_2gl_3gl_3$), ‘TM-1’ (a standard upland line with pigment gland genotype of $Gl_2Gl_2Gl_3Gl_3$) and *G. bickii* etc., were also used in this experiment (Table 1). All materials were inbreeding ones with continuous selfing more than 20 generations.

(ii) Cross method. Cross and backcross was made by the conventional crossing method. Flowers of the amphidiploid of (*G. arboreum*×*G. bickii*) F_1, whose bracts just came out were emasculated and covered with special waxed tubes, and then pollinated by selfed flowers from respective male parents at 8 to 10 o’clock in the next morning. All pollinated flowers were treated with one or two drops of mixture of 25 mg/L gibberellin and 25 mg/L NAA at the same time of pollination to prevent the bolls from shedding. Hybrids progenies were recurrently backcrossed to male parent upland cotton cultivars in the same way as indicated above. F_1 hybrid seeds for genetic research were planted in Hainan Province in the winter season to reproduce F_2 population.

(iii) Observation of pigment glands. Seeds from tri-specific hybrids and parent cultivars were delinted, and then soaked at 30℃ water for 24 h. Seed coats were removed after imbibition, and kernel glands were counted subsequently under a zoom stereomicroscope. Developmental dynamics of cotyledon pigment was observed every 12 h after seed germination. The density and size of pigment gland of each organs were determined and measured with zoom stereomicroscope as well, the former was determined by 4×20 times photograph scope frame as a unit, and then converted into the number of pigment glands per area (the number of pigment glands in the photograph scope frame/0.52×100 = the number of pigment gland/cm^2). The diameter of gland was measured directly as the size with a micrometer under a microscope.

(iv) Determination of gossypol content. Cotton samples at the same age were sampled, dried, and then grinded, filtered for further use. The gossypol contents for each sample were determined according to the aniline colorimetric method as described by Smith[14].

(v) Identification of agronomic and economic characters. The comparative experiment of yields was arranged in randomized design with three rows of each treatment and replicated thrice. ‘Siman3’, an extended cotton cultivar was used as check. Fifty normal open-bolls were sampled before harvesting for investigation and determination of agronomic and fiber properties. Fiber quality was determined by the Detection Center for Cotton Quality of MOA in Anyang, Henan. Disease resistance was identified by the Plant Protection Lab in Cotton Research Institute, the Chinese Academy of Agricultural Sciences.

2 Results and analysis

(i) The breeding process of ‘ABH-0318’. In 1993, the tri-specific hybrid with delayed pigment gland morphogenesis was obtained by crossing the amphidiploid of (*G. arboreum*×*G. bickii*) F_1 as female parent with upland cotton germplasm with pigment gland genotype of $Gl_2Gl_2gl_3gl_3$ in Anyang, Henan Province. Out of a total of 74 flowers pollinated, 9 hybrid bolls (with 1—4 seeds in each boll) were harvested with a successful cross rate up to 12.2%. In total, 17 well-developed hybrid seeds were obtained and five of them were planted in the field. The remaining seeds were used for analysis and observation of pigment glands, and determination of gossypol content.

Backcrossed seeds were obtained by backcrossing $Gl_2Gl_2gl_3gl_3$ as recurrent parent with the tri-specific hybrid. Afterwards, the seeds whose kernel had a few or no pigment glands were selected to grow in the field through observation of seed kernel. Further gland characters in plant organs were observed and subsequently those glanded plants were screened to continuous backcrossing with recurrent parent. During the backcross breeding program, most plants in BC_1-BC_3 generations were highly sterile and no selfed seeds were produced. By the backcrossing, partial BC progenies had been produced and about 12.2%—18.1% of individuals carried the target trait. There were 5.0%—33.3% of fertile plants in the generations after BC_5, and the staining test of pollens showed that more than 80% of pollens were fertile. But no glandless seeds were produced in the selfed or backcross progenies of fertile plants, which indicated that the target trait had been lost in these fertile plants. In contrast, the individuals carrying the target trait were all sterile and only 2.4%—10.8% of pollens in these plants were stained.

By continuing backcrossing and enlarging the backcross population, two fertile plants with target trait were subsequently obtained in the BC_8 generation. After selfing for two generations, a new upland cotton germplasm 'ABH-0318' with delayed pigment gland morphogenesis trait was eventually obtained in 2002.

'ABH-0318' was a medium maturity upland cotton germplasm with the growth period of 130—135 d. The average lint yield before frost was 978.5 kg/ha which accounts for about 71.1% of the lint yield in extending upland cotton cultivar, and there were 11.5 bolls in average per plant, with the average boll weight of 5.4 g and ginning turnout of 36.8%. The fiber quality of 'ABH-0318' was good and its average staple length was 29.3mm, with a uniformity of 83.1%, strength 32.4 cN/tex, elongation 6.5%, and micronaire reading 4.5. The disease attacking percentage and disease index of Fusarium wilt in ABH-0318 were 24.4% and 8.6, and that of Verticiilium wilt was 48.7% and 25.5, respectively. There were no significant differences between ABH-0318 and the ordinary upland cotton cultivars in other stress tolerance.

(ⅱ) The characteristic of ABH-0318.

(1) Pigment gland expression. No pigment glands were observed in dormant seeds of line ABH-0318 by naked eyes, but under zoom of stereomicroscope, a few small glands whose diameter was 1/3 of that upland cotton could be found along the cotyledon edges. The gland expression in the dormant seeds of ABH-0318 was similar to that of its donor parent, the amphidiploid of (*G. arboreum*×*G. bickii*) F_1, but obviously different from that of upland cotton recurrent parent (Table 2). However, the pigment glands could be observed on cotyledons of ABH-0318 12 h after germination, and 4 d after germination it was noticed that the gland expression in the cotyledons of HAB-0318 was approximately the same as that of *G. bickii,* recurrent parent of *G. hirsutum* and the amphidiploid of (*G. arboreum*×*G. bickii*) F_1, but lower than that of TM-1.

The gland expressions on the main plant parts of ABH-0318 were similar to that of cotyledons 4 d after seed germination (Table 3). Comparing with TM-1, ABH-0318 had denser but smaller pigment glands in the leaves, bracts and calyx, similar ones in boll shells, and much scarcer ones in the petals. The density and size of pigment glands in the plant parts of ABH-0318 were smaller than its parent (*G. bickii*), except for petals. But it was quite similar to that of amphidiploid of (*G. arboreum* ×*G. bickii*) F_1 and its upland cotton recurrent parent. Obviously the plants of ABH-0318 were typical glanded cotton type, although there was slightly difference in pigment gland expression on various plant organs.

(2) Gossypol contents. The determination of gossypol contents presented in Table 4 show that the gossypol content in the seeds of ABH-0318 was only 0.0175%, much lower than that in ordinary glanded upland cotton, and lower than the gossypol content standards in food as established by WHO/FAO (0.02%—0.04%), implying that it is of typical low gossypol cotton type. However, the gossypol contents in the cotyledons after the seed germination, as well as other main plant parts of the ABH-0318 were as high as or even higher than upland cotton cultivars, belonging to the high gossypol cotton types.

Table 2 Gland expressions in the seeds of ABH-0318 and its cotyledons 4 d after germination

Materials	Density/glands • cm^{-2}		Size/μm	
	Kernel	Cotyledon	Kernel	Cotyledon
ABH-0318	56.6±21.3	107.5±30.3	31±3.2	106±11.4
G. bickii	0	104.0±8.9	–	97±8.5
AAGG	55.8±23.2	115.5±12.7	30±2.7	110±10.8
$Gl_2Gl_2gl_3gl_3$	187.2±22.8	101.5±21.4	132±10.2	102±10.5
TM-1(CK)	222.7±25.7	184.6±17.4	135± 9.4	129±13.0

Table 3 The density and size of the glands on the main plant parts of ABH-0318[a)]

Material		Leaf	Bract	Calyx	Petal	Boll shell
ABH-0318	GD	137.7±12.4	141.0±16.7	172.3±17.1	84.0±18.3	81.00±14.3
	GS	138.0±15.4	155.0±13.1	187.0±14.3	81.0±7.40	166.0±11.9
G. bickii	GD	200.4±26.7	211.0±18.3	195.6±13.6	0	171.3±16.4
	GS	245.0±22.6	289.0±26.7	207.0±20.0	–	241.0±21.4
AAGG	GD	157.0±15.8	139.6±10.2	162.8±16.1	87.0±10.4	99.00±13.0
	GS	180.0±17.6	250.0±22.7	190.0±14.2	81.0±8.40	290.0±22.1
$Gl_2Gl_2gl_3gl_3$	GD	135.3±11.4	144.0±14.5	158.2±15.8	81.0±13.4	101.0±14.6
	GS	157.0±14.4	150.0±12.7	182.0±16.1	83.0±7.60	123.0±11.4
TM-1	GD	60.00±7.20	81.30±11.4	126.0±13.8	112.0±11.3	89.40±8.30
	GS	146.0±15.3	215.0±16.7	188.0±16.4	105.0±9.70	176.0±17.1

a) GD as the gland density (gland • cm^{-1}), GS as the gland size (gland diameter, μm).

(iii) Cytogenetics observation. Cytological observations indicated that there were a large number of univalents, as well as many multivalents at the meiosis MI of PMC in trispecific hybrids (Table 5). The chromosome configuration was $2n = 52 = 41.15 \text{ I} + 4.38 \text{ II} + 0.55 \text{ III} + 0.04 \text{ IV}$, suggesting that the pollen sterility of the tri-specific hybrid was due to the abnormal chromosome behavior in the meiosis due to the typical interspecific hybrid. With an increase in backcross generations, the chromosome configuration in PMC meiosis was tending towards normal. In advanced generation BC_8, most chromosomes could pair to form bivalents normally, although the chromosome number in the backcrossing generations was 49~55, being aneuploid types, especially for those with target trait. Chromosome pairing in PMC of the backcrossed plants was much better since BC_5 generation (there were some fertile plants in the backcrossed population after BC_5) and the fertile individuals had 52 chromosomes already, but the sterile ones had still a different chromosome number (from 49 to 54) in different plants (Table 5). It should be pointed out that the seeds produced from fertile plants after BC_5 generation were all glanded ones, while there were about 10% of the seeds having the target trait in the progenies produced from sterile plants by backcrossing, which demonstrated that the chromosomes or chromosome segments of *G. bickii* had already been lost in the fertile euploid plants. Although there were a certain number of univalents and multivalents at the meiosis MI in the PMC, most of the chromosomes in ABH-0318 plants could pair normally to form the bivalents and more than 30% of the bivalents were cycle ones with bivalents crossover value of 1.47. The chromosome configuration of ABH-0318 was $2n = 52 = 4.43 \text{ I} + 23.38 \text{ II} + 0.19 \text{ III} + 0.06 \text{ IV}$. At meiosis anaphase I, chromosomes could move towards the two poles equally in most PMC, and then normally fertile tetra-spores and pollen grains were eventually formed, showing that the fertility of ABH-0318 was recovered.

(v) Genetic analysis of pigment gland characters. The pigment gland expressions on the seeds and cotyledons 4 days after germination in the intraspecific hybrid F_1 and F_2, which were produced by crossing ABH-0318 with the upland germplasms with different pigment gland genes, were investigated, and the results were shown in Tables 6 and 7.

Table 4 The gossypol contents in the main parts of plant ABH-0318 and its parents (%)

Materials	Kernel	Cotyledon	Leaf	Bract	Petal	Boll shell
ABH-0318	0.0175±0.0002	0.1505±0.0049	0.1274±0.0035	0.1035±0.0095	0.0245±0.0017	0.1600±0.0077
G. bickii	0.0025±0.0001	0.1870±0.0032	0.1324±0.0017	0.1361±0.0027	0.0160±0.0016	0.1451±0.0011
AAGG	0.0147±0.0002	0.1934±0.0018	0.1274±0.0014	0.1450±0.0126	0.0275±0.0029	0.2100±0.0090
$Gl_2Gl_2gl_3gl_3$	0.9481±0.0123	0.1844±0.0024	0.1527±0.0026	0.1210±0.0044	0.1800±0.0285	0.2010±0.0088
TM-1(CK)	1.0471±0.0244	0.1763±0.0018	0.1414±0.0009	0.1312±0.0009	0.1725±0.0021	0.2103±0.0010

Table 5 The chromosome configuration at PMC meiosis MI of HAB-0318 and its breeding generations

Materials	Chromosome configuration					Bivalent cross over value	Count cells
	I	II	III	IV	Chromosome number		
Trispecific hybrid	41.45	4.38	0.55	0.04	52.0	1.19	56
BC_1	21.60	15.00	0.63	0.03	53.6	1.16	68
BC_2	7.11	22.82	0.25	0.04	53.6	1.11	76
BC_3	7.36	21.38	0.77	0.16	53.1	1.16	87
BC_4	6.01	22.82	0.55	0.03	53.4	1.15	78
BC_5-BC_8 fertile plant	5.60	22.93	0.11	0.05	52.0	1.28	168
BC_5-BC_8 sterile plant	6.16	22.68	0.14	0.06	52.2	1.33	145
ABH-0318	4.43	23.38	0.19	0.06	52.0	1.47	120

Table 6 The gland expression on the hybrids between ABH-0318 and different gland genotype

Combinations	Genotype of testing parent	Expression of glands
ABH-0318×TM-1	$Gl_2Gl_2Gl_3Gl_3$	Dense glands in seeds and cotyledons
TM-1× ABH-0318	$Gl_2Gl_2Gl_3Gl_3$	Dense glands in seeds and cotyledons
ABH-0318×ZMS_{13}	$gl_2gl_2gl_3gl_3$	Glandless seeds, a few small glands in cotyledons
ZMS_{13}× ABH-0318	$gl_2gl_2gl_3gl_3$	Glandless seeds, a few small glands in cotyledons
ABH-0318×$Gl_2Gl_2gl_3gl_3$	$Gl_2Gl_2gl_3gl_3$	A few small glands in seeds, dense glands in cotyledons
$Gl_2Gl_2gl_3gl_3$× ABH-0318	$Gl_2Gl_2gl_3gl_3$	A few small glands in seeds, dense glands in cotyledons
ABH-0318×$gl_2gl_2Gl_3Gl_3$	$gl_2gl_2Gl_3Gl_3$	Dense glands in seeds and cotyledons
$gl_2gl_2Gl_3Gl_3$×ABH-0318	$gl_2gl_2Gl_3Gl_3$	Dense glands in seeds and cotyledons

Table 7 The result of genetic analysis for glands of ABH-0318 (hybrid F_2)

Combinations	Gland expression in F_2 population(kernel/cotyledon)				χ^2	P
	Dense/dense	Dense/rare	Rare/dense	Rare/rare		
ABH-0318×TM-1	212	2	48	3	0.1083(13∶3)	0.50—0.90
ABH-0318×ZMS_{13}	0	0	59	201	0.7385(1∶3)	0.25—0.50
ABH-0318×$Gl_2Gl_2gl_3gl_3$	77	5	246	6	0.5868(3∶1)	0.25—0.50
ABH-0318×$gl_2gl_2Gl_3Gl_3$	184	0	20	46	1.3067(12∶1∶3)	0.50—0.90

Table 6 shows that the pigment gland characters in the hybrid F_1 produced by crossing ABH-0318 with TM-1 and $gl_2gl_2Gl_3Gl_3$ were similar to that of the tester parent (namely both hybrid seeds and cotyledons possessed dense pigment glands). However, when ABH-0318 was crossed with glandless upland cotton ZMS_{13}, the hybrid seeds were glandless completely, but there were only a few small glands appearing along the edges of cotyledons or leaf venations, which indicated that the expression of pigment gland in hybrid F_1 had been weakened greatly. However, the pigment glands in seeds and cotyledons of the hybrid F_1 ABH-0318×$Gl_2Gl_2gl_3gl_3$ were similar to that in ABH-0318. The experiment showed that there was no significant difference in gland behavior among each reciprocal combination. Thus it could be concluded that the gene from *G. bickii*, controlling the gland trait of ABH-0318 was dominant or dominant epistatic over one of the upland cotton pigment gland genes (Gl_2), but it was recessive or recessive epistatic to another gland gene (Gl_3). The glandless genes in upland cotton had a strong weakening effect on the gland expression in the plant of ABH-0318. These results were consistent with that obtained from the interspecific hybridization we did before[15].

From breeding program and the investigation on gland character in F_2, it was deduced that there were two pairs of genes controlling the delayed pigment gland morphogenesis trait of ABH-0318. One of them was derived from glandless recessive gene (gl_3gl_3), provided by the recurrent parent during the successive backcrossing, whereas the other was from *G. bickii.* Since there were not any glanded seeds separated in F_2 generation in the combination produced by crossing ABH-0318 with recessive glandless cotton, and segregation ratio of glandless and glanded cotyledons was in accordance with the genetic law of 3∶1. It was presumed that the gland gene from *G. bickii* was allelic to Gl_2 gene. Meanwhile, the pigment gland segregation in the kernels of hybrids F_2 produced by crossing ABH-0318 with recurrent parent was also fit to one gene separation law, and the gene from *G. bickii* was dominant over Gl_2 gene. If gene from *G. bickii* was assumed as Gl_2^b, we came to the conclusion that the pigment gland genotype of ABH-0318 was $Gl_2^bGl_2^bgl_3gl_3$, and Gl_2^b was dominant over Gl_2, but it was recessive epistatic to Gl_3. In other genetic experiments, combinations produced by crossing ABH-0318 with TM-1, $Gl_2Gl_2Gl_3Gl_3$ and $gl_2gl_2Gl_3Gl_3$, were also in accordance with the genetic law of two-gene interaction, further suggesting that the pigment gland genotype of ABH-0318 was $Gl_2^bGl_2^bgl_3gl_3$.

3 Discussion

The delayed pigment gland morphogenesis traits in wild diploid cotton species such as *G. bickii* were an important character in cotton breeding. Muramoto[16] first obtained the allotriploid by crossing *G. hirsutum* with *G. sturtianum* and a fertile allohexaploid of (*G. hirsutum* × *G. sturtianum*) F_1 was subsequently developed through chromosome-doubling. Dilday[17] discovered one cotton plant with the trait of glanded leaves and bolls, but glandless embryos in the population of the allohexaploid. This special plant was then used as donor parent in crossing and backcrossing with TM-1, and noticed that the pentaploid generation in BC_1 still had the character of glandless seed and glanded plant. Altmam et al.[18] overcame the fertility barrier of BC_1 by using ovule or embryo culture techniques and sequentially obtained BC_2, BC_3 and BC_4 generation. The seeds of most individual plants in these generations were glandless, and glanded seeds separated only in BC_3 and BC_4. Rooney et al.[19] screened 4 monosomic addition lines ($2n$ = 53) with a chromosome of *G sturtianum* from the backcrossing population. By crosses of *G. arboreum* and *G. sturtianum*, Mergeai[20] obtained amphidiploid F_1 of *G. arboreum* × *G. sturtianum* with the glandless seed and glanded plant trait, which were sequentially used as bridge material in transferring the target trait into upland cotton[21−23]. Additionally, He et al.[24] studied the hybrid F_1 between glandless upland cotton and *G. bickii*. Kulkarni et al.[25] made a research on interspecific hybridization of *G. australe* and *G. herbaceum*. However, the upland cotton germplasm with target characters had never been developed because of the distant genetic relationship between Australian wild species and cultivated upland cotton, although there were so many research reports on interspecific hybridization. By crossing *G. arboreum* with *G. bickii*, an amphidiploid of (*G. arboreum* × *G. bickii)* F_1 with the delayed pigment gland trait was successfully obtained by the method of chromosome duplication[13]. In this experiment, the amphidiploid served as a donor parent to cross and recurrent backcross with an

upland cotton germplasm, $Gl_2Gl_2gl_3gl_3$. A new upland cotton germplasm, ABH-0318, with the delayed gland morphogensis trait of *G. bicki* was eventually developed using the method of directed screening and utilization of special gene interaction between the pigment gland genes of *G. bickii* and upland cotton. The development of this special germplasm evidently laid a foundation for breeding of new cotton cultivars with glanded plant and low gossypol seeds.

During the transformation program, although there were about 10% of the individuals with the target trait in the backcrossing population, they were all aneuploid plants with high sterile. Fertile euploid offsprings were not produced until BC_5 generation. However, no individuals with target trait were found in either selfed or backcrossed progenies from fertile plants. In contrast, they just appeared in the sterile aneuploid progenies. This consequence was consistent with the result of ref. [19]. It was hence deduced that the fertile euploid plants did not contain any chromosomes or chromosome fragments of *G. bickii*. Therefore, sterile aneuploid plants with target characters were selected to continuously backcross with the recurrent parent, in order to produce a larger backcross population in this experiment. Two fertile individuals with target character were eventually obtained in BC_8 and the results of cytological observations show that there were 52 chromosomes in these plants. It could be concluded that the probability of exchange and recombination of chromosomes was quite low because of the distant genetic relationship between parents in interspecific hybridization. However, it would be possible to develop new germplasm with target characters, as long as we enlarge the backcross population and continue recurrent crossing. In addition, in this method of transformation, an upland cotton of $Gl_2Gl_2gl_3gl_3$ was used as recurrent parent to cross and backcross with the amphidiploid of (*G. arboreum*×*G. bickii*) F_1 in this experiment. Thus, the interspecific hybrid expressed completely the delayed pigment gland morphogenesis traits of *G. bickii*, and the ratio of the plant with target trait was relatively high in the backcross progenies, which evidently made it more convenient to select and maintain target characters in the offspring. The results approved the importance of the upland cotton parent in the introgression of delayed pigment gland morphogenesis trait of *G. bickii*.

The results of cytogenetics investigation demonstrated that the pollens of ABH-0318 were fertile and the chromosome number was 52, but the chromosomes were not able to pair normally in PMC meiosis. There were still some univalents and multivalents at the meiosis MI. This might be due to the absence of coordination when chromosome fragments of *G. bickii* entered into genome of *G. hirsutum*, which was a common problem in the interspecific hybridization. Additionally, there were still a few glands in dormant seeds and the gossypol content in the kernels was higher than that of *G. bickii* although it was much lower than that of glanded upland cotton, while glands in plant of ABH-0318 were smaller in size than that in *G. bickii*. It might be due to the possibility that the material only hold some main genes that controlled the delayed pigment gland morphogenesis trait and partial of modifying genes had not been transferred. It was also possible that a certain gene interaction between gland genes of upland cotton and delayed pigment gland morphogenesis gene of *G. bickii* weakens its expression in this material. Therefore, even though the genetic characteristics of ABH-0318 had been relatively stable, it was still necessary to improve the delayed pigment gland morphogenesis trait in the germplasm, by the method of screen, strain hybridization, etc.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant No. 30270845) and the National High Technology Research and Development Program of China (Grant No. 2002AA207005)

References

1. Gao, Z. C., Li, J. F., Jiang, Y. X., Glandless cotton, a good quality protein resource, China Cotton, 1984, 6: 19—21.
2. Bushr, A., Low cost protein from cottonseed, Econ. Bot., 1973, 27: 137—140.
3. 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.
4. McMichael, S. C., Glandless boll in Upland cotton and its use in the study of natural crossing, Agron. J., 1954, 46: 527—528.
5. McMichael, S. C., Hopi cotton, a source of cottonseed free of gossypol pigments, Agron. J., 1959, 51: 630.
6. McMichael, S. C., Combined effects of the glandless genes gl_2 and gl_3 on pigment glands in the cotton plant, Agron. J., 1960, 52: 385—386.
7. McMichael, S. C., Selection for glandless seeded cotton plants, Crop Sci., 1969, 9: 518—520.
8. Afifi, A., Bary, A. A., Kamel, S. A. et al., Bahtim 110, a new strain of Egyptian cotton free from gossypol, Emp. Cot. Gr. Rev., 1966, 43(2): 112—120.
9. Bottger, G. T., Sheehan, E. T., Lukefahr, M. J., Relation of gossypol content of cotton plants to insect resistance, J. Econ. Entomol., 1964, 57: 283—285.
10. Elliger, C. A., Chan, B. G., Waiss, A. C. Jr., Relative toxicity of minor cotton terpenoid compared to gossypol, J. Econ. Entomol., 1978, 71(2): 161—164.
11. Jenkins, J. N., Maxwell, F. G., The comparative preference of insects for glanded and glandless cotton, J. Econ. Entomol., 1966, 59(2): 352—356.

12. 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.
13. Zhang, B. Q., Li, B. L., Wan, L. M. et al., Synthesis of allotetraploid cotton with [AG] complex chromosome set, Chinese Science Bulletin, 1994, 39(7): 644—648.
14. Smith, F. H., Determination of gossypol in leaves and flower buds of *Gossypium*, J. Amer. Oil Chem. Soc., 1971, 44: 267—269.
15. Zhu, S. J., Ji, D. F., Inheritance of the delayed gland morphogenesis trait in Australian wild species of *Gossypium*, Chinese Science Bulletin, 2001, 46(14): 1168—1173.
16. Muramoto, H., Hexaploid cotton: Some plant and fiber properties, Crop Sci., 1969, 9: 27—29.
17. Dilday, R. H., Development of a cotton plant with glandless seeds and glanded foliage and fruiting forms, Crop Sci, 1986, 26: 639—641.
18. Altman, D. W., Stelly, D. M., Kohel, R. L., Introgression of glanded-plant and glandless-seed trait from *Gossypium sturtianum* Willis into cultivated upland cotton using ovule culture. Crop Sci., 1987, 27(5): 880—884
19. Rooney, W. L., Stelly, D. M., Identification of four *Gossypium* monosomic alien addition derivatives from a backcrossing program with *G. hirsutum*, Crop Sci., 1991, 31(2): 337—341.
20. Mergeai, G., New perspectives concerning the methodology to be used for introgression of the glanded plant and glandless seed character in cultivated cotton (*Gossypium hirsutum* L.), Cotton Fibres Trop., 1992, 47(2): 113—118.
21. Bi, I. V., Baudoin, J. P., Mergeai, G., Cytogenetics of the 'glandless-seed and glanded-plant' trait from *Gossypium sturtianum* Willis introgressed into upland cotton (*Gossypium hirsutu*m L.). Plant Breeding, 1998, 117(3): 235—241.
22. Bi, I. V., Baudoin, J. P., Hau, B., Mergeai, G., Development of high-gossypol cotton plants with low-gossypol seeds using trispecies bridge crosses and in vitro culture of seed embryos, Euphytica, 1999, 106(3): 243—251.
23. Ahoton, L., Lacape, J. M., Baudoin, J. P. et al., Introduction of Australian diploid cotton genetic variation into upland cotton, Crop Science, 2003, 43 (6): 1999—2005.
24. He, J. X., Sun, C. W., A Scheme for Introgression of Delayed Gland Morphogenesis Gene from wild *Gossypium bickii* into cultivated upland cotton (*G. hirsutum*), Acta Genetica Sinica, 1994, 21(1): 52—58.
25. Kulkarni, V. N., Khadi, B. M., Sangam, V. S., Pre-breeding efforts for low gossypol seed and high gossypol plant in *G. herbaceum* L. cotton utilizing *G. australe* Mueller, Current Science, 2002, 82(4): 434—439.

(Received August 31, 2004, accepted November 3, 2004)