NOTES

# Inheritance of the delayed gland morphogenesis trait in Australian wild species of *Gossypium*

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**Abstract** **Five Australian wild cotton species with the delayed gland morphogenesis trait, as well as *G. arboreum*, *G. davidsonii* and four different gland genotypes of *G. hirsutum*, $Gl_2Gl_2Gl_3Gl_3$, $Gl_2Gl_2gl_3gl_3$, $gl_2gl_2Gl_3Gl_3$, and $gl_2gl_2gl_3gl_3$, were used in this experiment and 10 interspecific hybrids were obtained by the crossing among them. According to the gland expression on the seeds and plants of the interspecific hybrids, the inheritance of the delayed gland morphogenesis trait of Australian wild cotton species was opened out as follows: (ⅰ) the inheritance of the delayed gland morphogenesis trait was almost the same among the 5 Australian wild cotton species, and the gene or genes which controlled this trait may be located in the same loci. (ⅱ) The glandless seed trait of the Australian wild cotton species was dominant over the glanded seed trait of *G. arboreum*, a genome A species, and the seeds of interspecific hybrid $F_1$ between them were glandless. However, it was recessive over the glanded character of genome D species, *G. davidsonii*, and their $F_1$ was a typical glanded one. (ⅲ) The glandless seed trait of the Australian wild cotton species was recessive or incomplete dominant over the glanded cotton but dominant over the glandless cotton of *G. hirsutum*, and the glandless genes ($gl_2gl_2gl_3gl_3$) of upland cotton had great weakening effect on the glanded plant trait of the Australian wild cotton species on the other hand. For the two main glanded genes of upland cotton, the delayed gland morphogenesis trait of the Australian wild cotton species was dominant epistatic over glandless genes, $gl_2gl_2gl_3gl_3$, and one of the glanded genes, $Gl_2Gl_2$, but was recessive epistatic over the other glanded gene, $Gl_3Gl_3$. Therefore, it is much convenient to use $Gl_2Gl_2gl_3gl_3$ as the upland cotton parent in the interspecific hybridization and backcrossing afterward, in order to produce the upland cotton germplasm with glandless seeds and glanded plant trait.**

**Keywords: Australian wild cotton species, pigment gland, inheritance.**

Pigment gland, or gland in short, is a special structure in plants of *Gossypium* genus and its related species. Inside pigment glands there are gossypol and its derivatives that are toxic to human beings and monostomach animals but resistant to some cotton diseases and insects[1]. The glandless cotton varieties are susceptive to some cotton diseases and insects, as well as some animals such as rats and rabbits for lack of glands in the plants, although their seeds can be used as food or feed directly because of the low gossypol contents. The wild Australian diploid cotton species such as *G. bickii*, *G. sturtianum,* and *G. australe,* as well as some related plants of *Gossypium* such as some species in genus of *Gienfuegosia* have a very special trait called "delayed gland morphogenesis", in which the dormant seeds are glandless (low gossypol content) and the plant includes the cotyledons after germination are glanded[2]. Introgressing this special character from Australian wild cotton species into the cultivated upland cotton (*G. hirsutum*. L) could develop a new germplasm with glandless seeds and glanded plant trait, which can take the advantages of glanded and glandless cotton. For its glandless seed trait, it would be helpful for integrating utilization because of low gossypol content in the seeds, and for its glanded plant character, it would not lose any resistant ability to the diseases, insects and animals. As the special function on the utilization of cottonseed and the resistance to insects and diseases, it has become an important breeding target in many cotton production countries to transfer this trait into cultivated upland cotton.

Since Muramoto[3] first obtained the interspecific hybrid of (*G.hirsutum* × *G.sturtianum*) $F_1$ and its allohexaploid by chromosome doubling in 1969, many interspecific hybrids and their offspring between Australian wild cotton species and cultivated cotton were obtained by cotton breeders and geneticists[4−13]. However, the research work on inheritance of the delayed gland morphogenesis trait of Australian wild cotton species, as well as its genetic interaction with the known upland cotton gland genes, has not been reported up to now. Since 1994, we have successfully obtained 10 interspecific hybrids by the crossing among the 5 Australian wild cotton species, and crossing Australian wild cotton species with cotton species in different genomes and different upland cotton gland genotypes in Sanya, Hainan, where the climate is extremely good for interspecific hybridization. In this note, the gland expression on the seeds and plants of interspecific hybrids, the inheritance of delayed gland morphogenesis trait of Australian wild cotton species, and its genetic effects on the upland cotton gland genes were investigated and studied, which may provide a scientific foundation for introgression of this special trait into upland cotton.

## 1 Materials and methods

(ⅰ) Parents of interspecific hybridization. The parents used in interspecific hybridization were 5 Australian wild diploid cotton species, *G. sturtianum* J. H. Willis, *G. nandewarense* Fryxell, *G. australe* F.von Mueller, *G. nelsonii* Fryxell and *G. bickii* Prokhanov, which are grown in the Wild Cotton Garden, Cotton Research Institute, the Chinese Academy of Agricultural Sciences (CAAS), Sanya, Hainan. The upland cotton parents were TM-1, the standard upland cotton line with the gland genotype of $Gl_2Gl_2Gl_3Gl_3$; ZMS 22, the glandless cotton cultivar with

Table 1 The parents and crossing combinations of the interspecific hybridization

| Combinations | Abbreviation | Cross time | Location |
|---|---|---|---|
| *G. hirsutum* (TM-1, $Gl_2Gl_2Gl_3Gl_3$)×*G. sturtianum* | *G. hirsutum* 1×*G. sturtianum* | winter, 1994 | Sanya, Hainan |
| *G. hirsutum* ($ZMS_{22}$, $gl_2gl_2gl_3gl_3$)×*G. sturtianum* | *G. hirsutum* 5×*G. sturtianum* | winter, 1994 | Sanya, Hainan |
| *G. hirsutum* ($Gl_2Gl_2gl_3gl_3$) ×*G. sturtianum* | *G. hirsutum* 6×*G. sturtianum* | winter, 1994 | Sanya, Hainan |
| *G. hirsutum* ($gl_2gl_2Gl_3Gl_3$)×*G. sturtianum* | *G. hirsutum* 7×*G. sturtianum* | winter, 1994 | Sanya, Hainan |
| *G. bickii*×*G. nelsonii* | *G. bickii*×*G. nelsonii* | winter, 1995 | Sanya, Hainan |
| *G. bickii*×*G. australe* | *G. bickii*×*G. australe* | winter, 1995 | Sanya, Hainan |
| *G. arboreum* (QXXHZ)×*G. bickii* | *G. arboreum* 1×*G. bickii* | summer, 1999 | Hangzhou, Zhejiang |
| *G. australe* ×*G. nelsonii* | *G. australe* ×*G. nelsonii* | summer, 1999 | Hangzhou, Zhejiang |
| *G. sturtianum* ×*G. nandewarense* | *G. sturtianum* ×*G. nandewarense* | winter, 1994 | Sanya, Hainan |
| *G. davidsonii*×*G. australe* | *G. davidsonii*×*G. australe* | winter, 1995 | Sanya, Hainan |

the gland genotype of $gl_2gl_2gl_3gl_3$; Monodominant line 1, an upland germplasm with the gland genotype of $Gl_2Gl_2gl_3gl_3$; and Monodominant line 2, another upland germplasm with the gland genotype of $gl_2gl_2Gl_3Gl_3$. Other materials such as *G. arboreum*, *G. davidsonii*, and the allotetraploid of (*G. arboreum*×*G. bickii* )$F_1$ which was provided kindly by the Shanxi Agricultural University were used in this experiment as well. 10 interspecific hybrids were obtained in Sanya and Hangzhou during the period of 1994 — 1999, and listed in table 1.

(ii) Method of hybridization. The hybridization was done in the winter season of 1994 — 1995 in the Wild Cotton Garden, Cotton Research Institute, CAAS, Sanya, Hainan, as well as in the summer season of 1999 in Hangzhou, Zhejiang. According to the climate and the behavior of cotton pollination, the maternal parents in Hangzhou were emasculated in the afternoon, then the stigma after emasculation was covered with a stencil tube in order to avoid contamination with unneeded pollens. Pollination was carried out in the morning next day of emasculation. However, the situation in Sanya was quite different, the emasculation was carried out at 7—8 a.m., and the pollination was done at 3—4 p.m. in the same day of emasculation. After pollination, 1—2 drops of mixed solution of $GA_3$ and NAA (50 mg/L + 50 mg/L = 1∶1) were added to the inside of bracts to protect crossed boll from shedding. The hybrid seeds were planted in flowerpots both in Hangzhou and Anyang in May next year of hybridization. In the deep fall, the hybrid plants were moved into the greenhouse for living through the winter and backcrossing next year.

(iii) Observation of pigment gland. The hybrid seeds were soaked in water at 30℃ for 24 h, then dehulled before germination. After germination, the hybrid seeds were planted in the nursery and transplanted into flowerpots when the seedlings were grown up. The gland investigation was carried out during the plant growth period. The kernels of the hybrid seeds were observed for gland density and size before germination, and the plant parts and tissues with the same size and growing period were sampled and put into the petri dishes for moisture preservation. The gland investigation was carried out with a stereomicroscope, a photographic view frame with 4× 20 times was used as a counting unit for the gland density, then converted this counting number into gland number per unit area (glands/$cm^2$ = number of glands inside view frame/0.52×100), and for the gland size, the gland diameter was measured directly by a microscope with a micrometer.

## 2 Results and analysis

(i) Gland expression on seeds and plants of interspecific hybrid $F_1$ and $F_2$ among Australian wild cotton species. Four interspecific hybrid $F_1$, *G. bickii* × *G.nelsonii*, *G. bickii*×*G. australe*, *G. australe* ×*G. nelsonii*, and *G. sturtianum* ×*G. nandewarense*, were obtained by crossing among the 5 Australian wild cotton species in the winter seasons of 1994 and 1995 in the Wild Cotton Garden, Cotton Research Institute, CAAS, Sanya, Hainan and in the summer season of 1999 in the College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, Zhejiang, respectively. The hybrid seeds were planted in flowerpots in College of Agriculture and Biotechnology, Zhejiang University, and Cotton Research Institute, CAAS in May next year of hybridization. All the interspecific hybrids, except (*G. sturtianum*×*G. nandewarense*)$F_1$, could bloom in the 1st year of planting and a few $F_2$ seeds were produced afterward. The gland expression on the seeds and plants of the four interspecific hybrids and their parents were investigated and the results are shown in table 2.

It was very clear that all the seeds of 4 interspecific hybrid $F_1$ were glandless, and most of their plant parts were glanded as shown in table 2, i.e. the 4 interspecific hybrids had the delayed gland morphogenesis trait as their parents had. Anatomization of $F_2$ seeds found that there were no glands separation among the three interspecific hybrids, which was considered primarily that the character of glandless seeds and glanded plant of Australian wild cotton species may be controlled by the same genes or gene loci.

Table 2 The density (glands/cm$^2$) and size (μm) of the glands on the main parts of the interspecific hybridization[a)]

| Parents & Comb. | | Kernel | Cotyledon | Hypocotyl | Leaf | Petal | Boll shell |
|---|---|---|---|---|---|---|---|
| *G. sturtianum* | density | 0 | 144.7±13.1 | 167.5±18.4 | 195.5±21.0 | 189.3±61.4 | 199.2±33.7 |
| | size | — | 157.4±8.9 | 234.6±18.4 | 305.5±24.6 | 210.3±20.0 | 315.6±26.8 |
| *G. nandewarense* | density | 0 | 158.1±9.0 | 174.6±22.3 | 189.0±41.1 | 178.0±55.3 | 200.7±41.8 |
| | size | — | 144.7±10.7 | 240.6±17.3 | 289.7±32.1 | 190.8±24.4 | 300.8±31.2 |
| *G. australe* | density | 0 | 121.5±12.4 | 203.7±22.7 | 246.1±29.8 | 75.4±21.4 | 178.4±21.7 |
| | size | — | 94.6±6.8 | 150.6±10.4 | 220.7±21.4 | 100.3±11.1 | 300.6±31.7 |
| *G. nelsonii* | density | 0 | 132.7±13.1 | 213.4±24.6 | 254.1±31.5 | 0 | 201.3±33.6 |
| | size | — | 97.8±7.4 | 164.5±11.4 | 222.4±22.6 | – | 280.7±31.9 |
| *G. bickii* | density | 0 | 112.4±11.1 | 200.6±22.4 | 210.1±21.8 | 55.5±21.6 | 178.5±27.9 |
| | size | — | 90.7±6.9 | 155.4±13.7 | 241.1±31.1 | 85.6± 11.9 | — |
| (*G. bickii*× | density | 0 | 124.5±11.8 | 200.5±18.7 | 231.4±24.6 | 0 | — |
| *G. nelsonii*) $F_1$ | size | — | 93.2±11.4 | 160.2±20.8 | 221.1±25.2 | — | 260.2±33.1 |
| (*G. bickii*× | density | 0 | 133.1±16.8 | 201.7±26.1 | 240.6±30.1 | 66.7±11.6 | 184.1±23.9 |
| *G. australe*) $F_1$ | size | — | 99.9±15.1 | 175.6±20.1 | 200.1±22.7 | 70.4±17.3 | 280.3±31.1 |
| (*G. australe* × | density | 0 | 129.2±17.1 | 209.2±23.1 | 250.5±33.1 | 0 | 191.9±21.4 |
| *G. nelsonii*) $F_1$ | size | — | 95.5±14.1 | 160.1±21.9 | 221.0±30.1 | — | 290.7±33.3 |
| (*G. sturtianum* × | density | 0 | 150.0±12.9 | 170.0±20.2 | 192.3±32.7 | — | — |
| *G. nandewarense*) $F_1$ | size | — | 150.0±22.0 | 237.1±20.0 | 280.0±31.9 | — | — |

a) The samples of hybrids were drawn from the plants growing in Anyang, and those of the wild cotton species from the plants in Hainan.

Investigation of gland expression on the main parts of interspecific hybrid plants showed that the gland behave on the flowers was extremely different among the 4 interspecific hybrids. In the two interspecific hybrids of (*G. bickii*×*G. nelsonii*) $F_1$ and (*G. australe* ×*G. nelsonii*) $F_1$, the petals of flowers were glandless like their paternal parent, *G.nelsonii*; and there were only a few glands on the base overlap parts of petals in (*G. bickii*×*G. australe*) $F_1$ as what *G. australe* and *G. bickii* had. The glands on most plant parts of the 4 interspecific hybrids were intermediate, and the density and size of the glands on these parts were closed to their parents.

(ⅱ) Gland expression on the interspecific hybrids $F_1$ of Australian wild cotton species with *G. arboreum* and *G. davidsonii.* The genome of cultivated upland cotton is AADD, which had been considered universally as an allotetraploid species derived from an interspecific hybrid between A genome species and D genome species. In order to study the genetic effect of the delayed gland morphogenesis trait of Australian wild cotton species on the gland genes of different genomes in upland cotton, the interspecific hybridization of Australian wild cotton species with the cotton species in A genome (*G. arboreum*) and D genome (*G. davidsonii* and *G. thurberi*) were carried out, and two interspecific hybrids of (*G. davidsonii*× *G. australe*) $F_1$ and (*G. arboreum*×*G. bickii*) $F_1$ were obtained in the winter season of 1995 in Sanya, Hainan and the summer season in Hangzhou, Zhejiang, respectively. Most of the plant characters and growth habits of (*G. davidsonii*×*G. australe*) $F_1$ were intermediate between two parents; however, the characters of branches, hair and flower pigment were inclined to paternal parent, and other characters such as flowering habit were leaned to maternal parent. The morphological characters of (*G. arboreum*×*G. bickii*) $F_1$ were similar to those described by Li et al.[6] Both of the two interspecific hybrids $F_1$ were sterile highly, no seeds were obtained by selfing or backcrossing with any parents for several years. The results of gland investigation for seeds and main plant parts of two interspecific hybrids and their parents are shown in table 3.

From table 3, we know that the seeds of (*G. arboreum*×*G.bickii*) $F_1$ and its allotetraploid were typical glandless one. Although (*G. arboreum*×*G. bickii*) $F_1$ was a new interspecific hybrid developed in Hangzhou, Zhejiang in 1999, this result was completely consistent with what Li et al.[6] got in Taigu, Shanxi, in 1980. Like the character of *G. bickii*, the seeds of (*G. arboreum*×*G. bickii*) $F_1$ and its allotetraploid were glandless, while the cotyledons after germination would show glands, although the emergence time of gland on the cotyledons was a little earlier than that of *G. bickii*. Thus it can be seen that the interspecific hybrid of (*G. arboreum*×*G. bickii*) $F_1$ and its allotetraploid had the delayed gland morphogenesis trait of *G. bickii* completely, and the glandless seed character of *G. bickii* was dominant over the glanded seed trait of *G. arboreum*. In this interspecific crossing combination, the delayed gland morphogenesis character of *G. bickii* was dominant one, which was consistent with the results obtained by Mergeai[12] in the interspecific hybrid of (*G. arboreum*×*G. sturtianum*) $F_1$ and its allotetraploid.

The results of gland investigation on the plant of interspecific hybrid $F_1$ showed that the gland density on the cotyledons was higher than that of two parents, being a super-parent inheritance trait; while the size of gland on the cotyledons was intermediate, inclined to the paternal

Table 3 The density (glands/cm$^2$) and size (μm) of the glands on the main parts of the interspecific hybrids $F_1$[a)]

| Parents & Comb. | | Kernel | Cotyledon | Hypocotyl | Leaf | Petal | Boll shell |
|---|---|---|---|---|---|---|---|
| | density | 275.0±47.0 | 114.0±9.7 | 142.5±13.7 | 44.7±3.8 | 107.3±10.8 | 89.1±9.1 |
| *G. arboreum* | size | 111.0±10.2 | 155.2±15.4 | 150.5±14.2 | 105.0±7.4 | 60.4±3.1 | 184.0±17.5 |
| | density | 231.5±21.4 | 157.4±19.7 | 160.8±19.7 | 110.4±17.5 | 200.4±29.7 | 253.4±21.2 |
| *G. davidsonii* | size | 157.6±17.5 | 170.3±20.1 | 171.3±22.4 | 180.3±19.4 | 100.4±10.3 | 285.4±31.4 |
| | density | 0 | 104.0±8.9 | 189.0±31.3 | 205.0±29.3 | 55.9±21.4 | 164.3±15.3 |
| *G. bickii* | size | — | 90.0±8.3 | 165.3±22.1 | 200.3±21.3 | 80.3±8.4 | 195.0±18.4 |
| | density | 0 | 131.1±13.7 | 221.7±21.6 | 251.1±29.1 | 143.4±31.4 | 209.5±23.4 |
| *G. australe* | size | — | 90.1±7.0 | 148.3±10.6 | 200.0±20.9 | 90.3±12.1 | 287.4±32.3 |
| (*G. arboreum*× | density | 0 | 180.5±21.0 | 166.5±19.3 | 100.1±21.1 | 66.3±14.4 | 99.0±13.3 |
| *G. bickii*) $F_1$ | size | — | 100.3±11.1 | 121.5±12.4 | 170.1±15.9 | 70.2±7.9 | 281.5±21.0 |
| (*G. arboreum* × | density | 0 | 170.5±17.4 | 152.5±13.8 | 78.4±14.9 | 170.2±21.0 | 212.0±20.0 |
| *G. bickii*) $F_1$, allotetraploid | size | — | 110.0±9.0 | 141.0±12.0 | 180.2±19.0 | 50.0±8.8 | 290.0±25.7 |
| (*G. davidsonii*× | density | 223.8±24.6 | 153.1±14.1 | 200.5±21.6 | 250.4±28.1 | 112.4±21.4 | 210.4±24.6 |
| *G. australe*) $F_1$ | size | 155.4±16.8 | 170.0±10.4 | 150.4±15.0 | 207.3±23.4 | 90.2±11.4 | 287.5±30.4 |

a) The bolls of (*G. arboreum*×*G. bickii* ) $F_1$ and (*G. davidsonii*×*G. australe*) $F_1$ were set by $GA_3$ treatment.

parent of *G. bickii*. The glands on the hypocotyls were intermediate between two parents in density, but smaller in size than that of two parents. So the gland size on the hypocotyls of interspecific hybrid was a negative super-parent inheritance trait. The density and size of the glands on the leaves and petals of the interspecific hybrid $F_1$ were intermediate, with a little lean to *G. bickii*. While the glands on the boll shells of interspecific hybrid $F_1$ were intermediate in density between two parents, but bigger than two parents on the other hand, it was another super-parent inheritance trait. So it is obvious that the gland inheritance of the interspecific hybrid plant of (*G. arboreum* × *G. bickii*) $F_1$ was very complex, and the expression of glands among the cotyledons, hypocotyls and boll shells of interspecific hybrid was variant greatly.

The glands on plants between (*G. arboreum*×*G. bickii*) $F_1$ and its allotetraploid were consistent basically, and the variability trend of gland expression among the tissues and parts of the plants was similar as well. On the same tissue or part of the interspecific hybrid plant, the gland density and size of (*G. arboreum*×*G. bickii*) $F_1$ were smaller than that of its allotetraploid, by 5%—10% approximately, except the petals. It may be related to the size of allotetraploid which was derived from (*G. arboreum*×*G. bickii*) $F_1$ by chromosome doubling, while the flowers of allotetraploid plant were much smaller than that of (*G. arboreum*×*G. bickii*) $F_1$, due to their closed pollination habit. So it is obvious that the increase of gene dosage of the delayed gland morphogenesis character of *G.bickii*, increased from one set genes of the interspecific hybrid $F_1$ to two set genes of allotetraploid, was no material effect on the gland genetic expression of the interspecific hybrid, the variation of gland density and size between (*G. arboreum*×*G. bickii*) $F_1$ and its allotetraploid were caused only by the change in size of plant, tissue and cells.

It was quite different from the gland expression of (*G. arboreum*×*G. bickii*) $F_1$, the seed of (*G. davidsonii*× *G. australe*) $F_1$ was a typical glanded one, and the density of glands on the kernels of this interspecific hybrid $F_1$ was 223.8 glands/cm$^2$, and the size of that was 155.4 μm, which were closed to that of *G. davidsonii*. So it is obvious that the glanded seed trait of *G. davidsonii* was dominant over the glandless seed trait of *G. australe*. In this interspecific crossing combination, the delayed gland morphogenesis character of *G. australe* was a recessive inheritance trait.

All the plant parts of interspecific hybrid (*G. davidsonii*×*G. australe*) $F_1$ were glanded. Among these plant parts, the gland densities of cotyledons, hypocotyls, leaves and boll shells were 153.1, 200.5, 250.4, and 210.4 glands/cm$^2$, respectively, which were intermediate between two parents, lean to paternal parent, *G. australe*; while that of petals was 112.4 glands/cm$^2$ only, smaller than two parents, it was a negative super-parent inheritance trait. The gland sizes of cotyledons, hypocotyls, leaves, petals, and boll shells were 170.0, 150.4, 207.3, 90.0, and 287.5 μm, respectively, which were intermediate between two parents, inclined to paternal parents, *G. australe*, except for cotyledons which lean to maternal parent, *G. davidsonii*. It should be pointed out that all the materials in table 3 were sampled from the plants growing in Anyang, Henan, where the flowers of *G. australe* were closed pollination, and the petals were very small. So the gland inheritance of (*G. davidsonii*×*G. australe*) $F_1$ plant was very complex, which may be affected by the environments and plant growth status, etc.

(iii) The genetic effect of gland genes of *G. hirsutum* on the delayed gland morphogenesis character of the Australian wild cotton species. Totally 295 flowers were emasculated and pollinated in Feb., 1994 in Wild Cotton Garden, Cotton Research Institute, CAAS, Sanya, Hainan,

and 8 hybrid bolls with seeds and 9 interspecific hybrid seeds were obtained, with a successful interspecific hybridization of 2.71%. The interspecific hybrid seeds of TM-1×*G. sturtianum*, $ZMS_{22}$×*G. sturtianum*, Monodominant line 1×*G. sturtianum*, and Monodominant line 2×*G. sturtianum* were 3, 3, 2, and 1, respectively. The hybrid seeds were planted in the beginning of May, 1995, in Anyang and Henan. All the seeds could grow into plants and flower in the same year of planting, but no seeds were obtained by selfing or backcrossing with *G. hirsutum*. The results of gland expression on the seeds and plants of interspecific hybrids are shown in tables 4 and 5, respectively.

Because of the insufficiency of the interspecific hybrid seeds, the results of gland density and size on kernels of interspecific hybrids in table 4 were rough results which were obtained by observation of the whole absorbed kernels under a stereomicroscope. However, it was still clear enough to find out the fast that the kernels of interspecific hybrid $F_1$ produced by crossing *G. sturtianum* with $ZMS_{22}$ and Monodominant line 1 were spotless (glandless) completely, and other interspecific hybrid seeds were glanded although the gland density and size of those interspecific hybrid $F_1$ were much less than that of normal glanded upland cotton. It is thus obvious that the gland genes of *G.hirsutum* had some effect on the gland expression of the interspecific hybrid seeds, there was a significant gene interaction between the gene or genes which control the delayed gland morphogenesis trait of *G. sturtianum* and gland genes of *G. hirsutum*. On the seeds, the genes of delayed gland morphogenesis trait of *G. sturtianum* are dominant epistatic over the glandless genes ($gl_2gl_2gl_3gl_3$) and one of the glanded genes ($Gl_2Gl_2$) of *G. hirsutum*, but recessive epistatic over another glanded gene, $Gl_3Gl_3$.

For the two interspecific crossing combinations with the glandless seeds trait, the glands on the cotyledons of the interspecific hybrid $F_1$ which derived from crossing between *G. sturtianum* and Monodominat line 1 could be seen gradually just a few hours after germination when they were presoaked for 24 h and dehulled, as the color of the glands became darker and darker. When the cotyledon was unfold, the size and color of the glands on the cotyledons and hypocotyls were almost the same as those of glanded upland cotton, i.e. the glandless seeds have been changed into glanded plant type. In addition, the time of gland expression on the cotyledons is also the same as that

Table 4 The gland expression on the kernels and the cotyledons of (*G. hirsutum*×*G. sturtianum*) $F_1$[a)]

| Material | Gland density/glands • $cm^{-2}$ | | Gland size/μm | |
|---|---|---|---|---|
| | kernel | cotyledon | kernel | cotyledon |
| *G.hirsutum* (TM-1, $Gl_2Gl_2Gl_3Gl_3$)×*G. sturtianum* | 118.0 ± 13.4 | 147.1 ± 10.1 | 96 ± 1.8 | 123 ± 12.4 |
| *G.hirsutum* ($ZMS_{22}$, $gl_2gl_2gl_3gl_3$)×*G. sturtianum* | 0 | 81.3 ± 24.3 | — | 92 ± 14.1 |
| *G.hirsutum* ($Gl_2Gl_2gl_3gl_3$) ×*G. sturtianum* | 0 | 124.2 ± 12.7 | — | 119 ± 13.7 |
| *G.hirsutum* ($gl_2gl_2Gl_3Gl_3$)×*G. sturtianum* | 109.9 ± 12.6 | 131.1 ± 14.7 | 101 ± 7.5 | 121 ± 13.1 |
| TM-1 | 222.7 ± 25.7 | 184.6 ± 17.4 | 135 ± 9.4 | 129 ± 13.0 |
| $Gl_2Gl_2gl_3gl_3$ | 121.3 ± 10.4 | 160.5 ± 10.3 | 128 ± 13.1 | 120 ± 11.4 |
| $gl_2gl_2Gl_3Gl_3$ | 110.5 ± 12.1 | 148.4 ± 10.5 | 125 ± 12.6 | 126 ± 13.4 |

a) $ZMS_{22}$ was glandless cotton and all parts of the plant were glandless, so its results were omitted in the table.

Table 5 The density (glands/$cm^2$) and size (μm) of the glands on the main parts of (*G. hirsutum*×*G. sturtianum*) $F_1$[a)]

| Material | | Leaf | Bract | Calyx | Petal | Boll shell |
|---|---|---|---|---|---|---|
| *G.hirsutum* (TM-1)× | density | 79.0±7.0 | 90.1±8.9 | 129.2±10.1 | 137.5±14.1 | 121.2±11.2 |
| *G. sturtianum* | size | 173±11.4 | 177±12.3 | 185±13.7 | 112±10.1 | 178±13.4 |
| *G.hirsutum* ($ZMS_{22}$)× | density | 40.1±7.2 | 56.2±12.1 | 89.4±12.7 | 89.5±13.8 | 97.4±10.1 |
| *G. sturtianum* | size | 88±11.4 | 112±14.6 | 107±9.8 | 66±6.5 | 131±13.4 |
| *G.hirsutum* ($Gl_2Gl_2gl_3gl_3$) × | density | 79.3±8.4 | 88.3±13.7 | 131.4±14.1 | 133.0±13.1 | 121.7±10.9 |
| *G. sturtianum* | size | 182±14.1 | 178±14.2 | 181±14.9 | 117±10.6 | 179±11.7 |
| *G.hirsutum* ($gl_2gl_2Gl_3Gl_3$)× | density | 80.1±10.4 | 77.3±11.4 | 99.1±12.6 | 126.0±13.1 | 132.1±11.8 |
| *G. sturtianum* | size | 154±11.2 | 175±10.1 | 179±14.7 | 112±9.8 | 187±15.1 |
| TM-1 | density | 60.0±7.2 | 81.3±11.4 | 126.0±13.8 | 112.0±11.3 | 89.4±8.3 |
| | size | 142±11.4 | 215±12.4 | 234±21.3 | 88±4.6 | 241±20.5 |
| $Gl_2Gl_2gl_3gl_3$ | density | 64.3±13.1 | 72.3±10.4 | 116.3±14.1 | 84.5±10.1 | 73.6±8.7 |
| | size | 140±14.1 | 141±14.0 | 199±20.1 | 72±8.7 | 141±14.0 |
| $gl_2gl_2Gl_3Gl_3$ | density | 58.3±11.4 | 72.0±11.1 | 121.8±14.7 | 80.4±9.8 | 72.0±11.1 |
| | size | 137±13.1 | 138±13.0 | 180±17.9 | 70±11.1 | 138±13.0 |

a) $ZMS_{22}$ was glandless cotton and all parts of the plant were glandless, its results were omitted in the table.

of *G. sturtianum*, which indicated that the delayed gland morphogenesis trait of *G. sturtianum* was expressed completely on this interspecific hybrid $F_1$. However, the gland on cotyledons of the interspecific hybrid produced by crossing *G. sturtianum* with $ZMS_{22}$ was less than that of TM-1, *G. sturtianum* and other interspecific hybrid with glanded upland cotton, although there were a few glands on the cotyledons and hypocotyls after germination. It is clear that the gland expression on the seedlings of interspecific hybrid was restrained by the upland cotton glandless genes, $gl_2gl_2gl_3gl_3$. For the two interspecific hybrids with $Gl_2Gl_3$ and $gl_2Gl_3$, although the seeds and plants of those interspecific hybrids were glanded, the glands on the kernels and cotyledons after germination were much less and smaller than that of their upland cotton parents, which indicated that the delayed gland morphogenesis trait of *G. sturtianum* was dominant incompletely over $Gl_2Gl_2Gl_3Gl_3$ and $gl_2gl_2Gl_3Gl_3$, and it may be affected by some quantity genetic components.

The expression of glands on the plant parts of interspecific hybrids was almost the same as that of glanded upland cotton, and the density and size of the glands were intermediate between two parent species, inclined to *G. hirsutum*. However, the glands on the interspecific hybrid plants produced by crossing with glandless upland cotton were less in density and smaller in size, compared with other 3 interspecific hybrid plants. It is very clear that the glandless genes of upland cotton have some weaken effects on the plant gland expression of *G. sturtianum*. It is concluded that there were some genetic interactions or gene dosage effects among the genes controlled the gland character in different cotton species.

## 3 Discussion

(ⅰ) The inheritance of the delayed gland morphogenesis in Australian wild cotton species. The inheritance character of the delayed gland morphogenesis of the Australian wild cotton species has not been discovered up to now. The results of Li et al.[6,7] showed that the delayed gland morphogenesis trait of *G. bickii* was dominant over the glanded trait of *G. arboreum*. Dilday[11] found a single plant with the glandless seeds and glanded plant trait from Muramoto's (*G. hirsutum*×*G. sturtianum*) $F_1$ allohexaploid population, and the special trait of this single plant was still expressed in its $BC_1$ generation produced by backcrossing this plant with *G. hirsutum* (TM-1), which suggested that the glandless seeds and glanded plant trait of this mutant plant was dominant one. Mergeai[12] obtained several interspecific hybrid $F_1$ by crossing *G. sturtianum* with *G. arboreum* and some wild species in genome D, and found that the (*G. arboreum*× *G. sturtianum*) $F_1$ had the glandless seeds and glanded plant trait, while that of(*G. thurberi*×*G. sturtianum*) $F_1$ and (*G. davidsonii*×*G. sturtianum*) $F_1$ was typical glanded one, which showed that the delayed gland morphogenesis trait of *G. sturtianum* was dominant over the gland trait of *G. arboreum*, and recessive over that of *G. thurberi* and *G. davidsonii*. However, the results of He et al.[5] showed that the delayed gland morphogenesis was dominant trait for the glandless upland cotton, but recessive one for the glanded upland cotton. According to the results of our experiment on the expression of glands on the seeds and plants of interspecific hybrids, the following outcomes were drawn primarily as follows: ⅰ) All the 5 Australian wild species of *Gossypium* have the trait of delayed gland morphogenesis, and the gene(s) that controlled this trait in different species might be located in the same loci. ⅱ) This special trait was dominant character over the species of genome A, but recessive one over the species of genome D. For allotetraploid upland cotton species with genome AD, this special trait was recessive or partial dominant over glanded cotton, but dominant over glandless ones, although the glandless genes of *G. hirsutum* could affect the gland expression on interspecific hybrid plants greatly. ⅲ) For the two main genes that controlled the gland character of *G. hirsutum*, $Gl_2$ and $Gl_3$, this special trait was dominant epistasis over $Gl_2$, and recessive epistasis over $Gl_3$. However, because of the restriction of the experimental materials, it is very difficult to get $F_2$ and offspring afterward for cotton interspecific hybridization. So the inheritance of delayed gland morphogenesis character of the Australian wild cotton species should be studied and approved furthermore.

(ⅱ) The method of introgressing delayed gland morphogenesis trait from Australian wild cotton species.

The delayed gland morphogenesis is a very important trait in cotton genetics and breeding, and to develop a cotton germplasm with glandless seeds and glanded plant trait is one of the most important cotton breeding targets. According to the results in this note, the gland genotypes of cultivated cotton species in the interspecific hybridization was a key technique in introgression of the delayed gland morphogenesis trait of Australian wild cotton species. If we used normal glanded cotton as cultivated parent to cross or backcross with the Australian wild cotton species, the hybrid seeds were glanded and the target trait was covered by the glanded genes of upland cotton, which may lead to the difficulty in selection of target character, sometimes the target character may be lost before the stabilization of hybrid in agricultural and economic characters. While the hybrid derived from crossing with glandless upland cotton, the gland expression on the interspecific hybrid plant was like those of intraspecific hybrid between glandless and glanded upland cotton although the hybrid seeds were glandless, and the expression of target trait was interfered by the glandless genes of upland cotton, which may lead to the difficulty in selection as well. If we used the Monodominant line 1($Gl_2Gl_2gl_3gl_3$) as up-

land cotton parent to cross or backcross afterward with Australian wild cotton species, the delayed gland morphogenesis trait was retained in the interspecific hybrid completely, the percentage of plants with target trait in the backcrossing offspring was relatively high. So it might be much easy to select the glandless seeds and glanded plant trait in the population of interspecific hybrids and keep this character in the offspring. Therefore, it is much more convenient to transfer the delayed gland morphogenesis trait from Australian wild cotton species into upland cotton using the Monodominant line 1 ($Gl_2Gl_2gl_3gl_3$) as the upland cotton parent in the interspecific hybridization and backcrossing afterward. At the same time, it is worthwile to study deeply the introgression and utilization of the gland trait of the Australian wild cotton species.

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