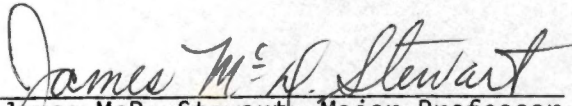
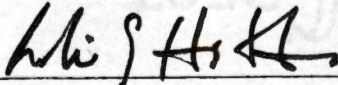
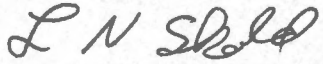


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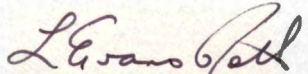
I am submitting herewith a thesis written by Katye Ross entitled "Pigment Gland Expression in Gossypium Interspecific Hybrids." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Science.


James McD. Stewart, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:


Vice Chancellor
Graduate Studies and Research

PIGMENT GLAND EXPRESSION IN GOSSYPIUM INTERSPECIFIC HYBRIDS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville



Katye Ross

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ABSTRACT

The pigment glands in cotton impart a degree of insect resistance to the plant, however, the terpenes in the glands also make the seeds toxic to non-ruminant animals. An ideal cotton plant would have many large glands in the foliage, but would have no glands in the seeds. While both of these traits occur individually in different wild Gossypium species, they do not occur in upland cotton. Gossypium sturtianum has a nearly glandless embryo that forms glands upon germination. The foliage glands of Gossypium anomalum are both large and numerous with the boll glands being especially large. The objective of this study was to examine the expression of these traits when the wild species are hybridized with cultivated cottons. A second objective was to determine, if possible, what type of inheritance controls the trait expression.

To evaluate the gland seed trait, three generations of seeds of hexaploid hybrids involving cultivated cotton and G. sturtianum were examined. A wide and continuous range in gland expression among seeds was noted, and in order to group seeds of similar phenotypic expression, four categories were established: very few glands (vfg), few glands (fg), several glands (sg), and glanded (gld). Selected seeds from each category in the first and second generations examined were germinated and grown to maturity. The gland densities in leaf, bract, and stem samples were examined on individual plants. The density of glands in the vegetative portions of the plant was independent of the seed gland category from which it descended.

Under field conditions seed set of the hybrids was very low and segregation ratios could not be determined. However, sufficient seed were obtained to establish that the inheritance of seed gland density is quantitative.

To evaluate the large foliage gland trait of G. anomalum, leaf gland expression in F_1 and F_2 generations of an intergenomic cross between Gossypium arboreum and G. anomalum was studied. The mean leaf gland density of F_2 plant samples was larger than the mean leaf gland density of F_1 and G. arboreum leaf samples. The range in leaf gland density was greater for the F_2 plants than for F_1 , G. arboreum, and G. anomalum leaf samples. The wide range observed among F_2 plants indicates quantitative inheritance for the trait.

The results of this study indicate the possibility of introgressing the near glandless trait of G. sturtianum and the large vegetative gland trait of G. anomalum into commercial varieties of cotton. Both traits appear to be quantitatively inherited and selection pressure for these traits in hybrids of wild and commercial cotton may ultimately result in the ideal commercial and agronomic goals of glandless seeds and heavily glanded vegetative parts.

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CHAPTER I

INTRODUCTION

Nearly all commercial cottons are characterized by the presence of pigment glands in all aerial parts of the plant and in the seed. These glands contain a variety of terpenoids, particularly gossypol, that discourage insect predation of the plant. However, because gossypol is a toxic substance, the otherwise high quality oil and meal that can be produced from cotton seed can not be consumed by humans and non-ruminant animals unless gossypol is detoxified. This is very expensive for the seed processing industry and lowers the nutritional value of the meal. Therefore, for maximum agronomic usage of commercial cotton, the vegetative portions should have a high density of large glands while the seed should be glandless.

Two wild Gossypium species that are recognized as sources for vegetative pigment gland enhancement and glandless seed are Gossypium anomalum Wawra ex Wawra & Peyritch and Gossypium sturtianum Willis, respectively. Through interspecific hybridization of these species with cultivated varieties of Gossypium hirsutum L., Gossypium barbadense L., and Gossypium arboreum L., introgression of the desired gland traits into commercial cottons has been made possible.

Very little work has been done in evaluating the gland expression of interspecific Gossypium hybrids. Therefore, the objectives of this study were:

1. Evaluate seed and vegetative gland expression in advanced generation hexaploid hybrids of G. hirsutum and G. barbadense with G. sturtianum;
2. Evaluate segregation of vegetative gland expression in the F_2 generation of a hybrid between G. arboreum and G. anomalum.



CHAPTER II

LITERATURE REVIEW

The Agronomic Importance of Pigment Gland Expression in *Gossypium*

Historically, cotton was produced for its fiber and the seed was a by-product. In the beginning of the twentieth century, however, cotton seed milling technology had advanced to such a state that a good edible oil could be obtained and used for many food products. The meal was used for cattle feed (51).

Today cotton seed is one of the leading oilseeds of the world and accounts for approximately one-tenth of the global production of edible vegetable oils and protein meals (23). However, because gossypol in cotton seed glands is toxic to non-ruminant animals (1, 6, 13, 19), it must be detoxified before the meal can be used for human or non-ruminant animal consumption.

Commercially the meal usually is heated before the oil is extracted so that gossypol binds to the protein (45). This is costly, requires a great deal of energy, and the protein quality of the meal is reduced by the chemical reaction between gossypol and the lysine in the protein (1, 6, 36).

The polyphenolic compound gossypol is a major component of the pigment glands in glanded cotton (2, 6) but it occurs only to a very limited extent in glandless cotton cultivars (9, 44). Glandless cotton seed requires approximately 50% less energy to process than glanded seed (52). For foodstuffs, glandless seed is superior to glanded

seed, yielding a high quality clear oil (50), and meal from which can be made protein products of high nutritive value (20, 50, 51). The demand for these high quality protein products as extenders for meats, cheeses, non-dairy protein beverages, and for fortification of low protein cereal based foods is well documented (8, 28, 29, 47, 51).

On the other hand, gossypol was first recognized as a source of insect resistance in Egyptian varieties of G. barbadense (42). Also, more recent studies (26, 27, 45) have demonstrated the toxicity of gossypol to insects when fed as part of a laboratory diet. Also, the preference of arthropods to feed on glandless G. hirsutum or G. barbadense rather than glanded varieties is well documented. Bottger et al. (7) showed that glandless lines of 'Acala' and 'Pima' were more susceptible than corresponding glanded lines to attack by beet armyworm, Spodoptera exigua; bollworm, Heliothis zea; black fleahopper, Spanogonicus albofasciatus; cutworms (undetermined spp.); pillbugs, Porcellio spp.; and grape colaspis beetle, Maecolaspis flavida. Jenkins (21) reported that the following serious cotton insects show a preference for glandless cotton over glanded cotton when given a choice: bollworm, Heliothis zea; tobacco budworm, Heliothis virescens; pink bollworm, Pectinophora gossypiella; and lygus, Lygus hesperus. Maxwell et al. (30) and Murray et al. (40) reported damage to glandless cotton by the striped blister beetle, Epicauta vittata, which is not normally known to attack glanded varieties of cotton.

Characterization of the Genus *Gossypium*

According to Fryxell (17), the genus *Gossypium* is comprised of 32 diploid and six tetraploid species. Within the 38 species eight genome groups are recognized. Classification of the species of *Gossypium* by genome formula, chromosome number, geographic origin, and use is shown in Table 1.

Originally Beasley (5) characterized the genomes of *Gossypium* into six groups on the basis of chromosome pairing in interspecific hybrids. The same letter of the alphabet was assigned to species usually giving fertile F_1 hybrids. Letters B, C, D, and E represent decreasing meiotic chromosome homoeology in F_1 hybrids involving an A genome and another species. With the exception of species containing both the A and D genomes, all others that have been examined are diploid with a haploid chromosome number of 13. The tetraploid AD genome species have a haploid chromosome number of 26. Of these, 13 chromosomes are large and closely related to the A genome, and 13 are small and closely related to the D genome (5, 18, 46).

The F genome was assigned by Phillips and Strickland (41) and the G genome was designated by Edwards and Mirza (14). Species containing the F or G genome do not follow the Beasley convention with respect to the degree of homoeology with the A genome. *Gossypium longicalyx* Hutchinson & Lee is the only species known to contain the F genome and *Gossypium bickii* Prokhanov is the only species known to contain the G genome. These two species each exhibit a degree of homoeology with the A genome that is distinct from the other genomes.

Table 1. Classification of the species of Gossypium.

Species	Genome formula	2n chromosome no.	Geographic origin	Use
<u>G. herbaceum</u>	A ₁	26	Africa	cultivated & wild
<u>G. arboreum</u>	A ₂	26	Africa	cultivated
<u>G. anomalum</u>	B ₁	26	Africa	wild
<u>G. triphyllum</u>	B ₂	26	Africa	wild
<u>G. capitata viridis</u>	B ₃	26	Cape Verde Islands	wild
<u>G. sturtianum</u>	C ₁	26	Australia	wild
<u>G. robinsonii</u>	C ₂	26	Australia	wild
<u>G. australe</u>	C ₃	26	Australia	wild
<u>G. nelsonii</u>	-	-	Australia	wild
<u>G. thurberi</u>	D ₁	26	Mexico & U.S.	wild
<u>G. amourianum</u>	D ₂₋₁	26	Mexico	wild
<u>G. harknessii</u>	D ₂₋₂	26	Mexico	wild
<u>G. klotzschianum</u>	D _{3-k}	26	Galapagos Islands	wild
<u>G. davidsonii</u>	D _{3-d}	26	Mexico	wild
<u>G. aridum</u>	D ₄	26	Mexico	wild
<u>G. raimondii</u>	D ₅	26	Peru	wild
<u>G. gossypoides</u>	D ₆	26	Mexico	wild
<u>G. lobatum</u>	D ₇	26	Mexico	wild
<u>G. trilobum</u>	D ₈	26	Mexico	wild
<u>G. laxum</u>	D ₉	26	Mexico	wild
<u>G. turneri</u>	-	-	Mexico	wild
<u>G. stocksii</u>	E ₁	26	Africa & Arabia	wild
<u>G. somalense</u>	E ₂	26	Africa	wild
<u>G. areysianum</u>	E ₃	26	Arabia	wild
<u>G. incanum</u>	E ₄	26	Arabia	wild
<u>G. longicalyx</u>	F ₁	26	Africa	wild
<u>G. bickii</u>	G ₁	26	Australia	wild
<u>G. populifolium</u>	-	-	Australia	wild
<u>G. pilosum</u>	-	-	Australia	wild
<u>G. costulatum</u>	-	-	Australia	wild
<u>G. cunninghamii</u>	-	-	Australia	wild
<u>G. pulchellum</u>	-	-	Australia	wild
<u>G. hirsutum</u>	AD ₁	52	Central America	cultivated
<u>G. barbadense</u>	AD ₂	52	South America	cultivated
<u>G. tomentosum</u>	AD ₃	52	Hawaii	wild
<u>G. mustelinum</u>	AD ₄	52	Brazil	wild
<u>G. lanceolatum</u>	AD ₅	52	Mexico	wild
<u>G. darwinii</u>	AD ₆	52	Galapagos Islands	wild

As indicated in Table 1 there are seven species for which the genome designations have not been cytologically determined.

Genome Contributions to Commercial Cotton Improvement

The A genome is responsible for the abundant fiber production on which the agronomic importance of commercial cotton is based. The four cultivated species are: Gossypium herbaceum L. (A₁), G. arboreum (A₂), G. hirsutum (AD₁), and G. barbadense (AD₂). Gossypium hirsutum, the AD genome species that comprises over 90% of the world's cultivated cotton, has been hybridized with species containing all other known genomes. Demol et al. (11, 12) showed how some of the wild diploid species can favorably influence important fiber quality traits and other agronomic traits when combined with G. hirsutum in a hexaploid hybrid. The B genome contributed to improved fiber length and drought resistance; the C genome increased micronaire, fiber strength, and ginning turnout; and the D and E genomes contributed to higher fiber strength.

The artificial introgression of agronomically useful traits from various genomes of Gossypium into the cultivated tetraploid species is well documented. Meyer (35) and Fryxell (16) reviewed much of this work. Some of the important characteristics transferred to cultivated cotton include hairiness from G. arboreum to G. barbadense (22); "D₂" smoothness from Gossypium armourianum Kearney to G. hirsutum (33); nectariless from Gossypium tomentosum Nuttall ex Seemann to G. hirsutum (34); and cotton rust resistance from G. anomalum to G. hirsutum (16).

The transfer of rust resistance from G. anomalum to G. hirsutum (16) illustrates the feasibility of using an intergenomic hybrid and artificial chromosome doubling as techniques for experimental introgression of a gene into a foreign genome. A hybrid was made between G. hirsutum and G. anomalum and the chromosome number of the resulting ADB triploid was doubled with colchicine to produce a fertile AADDBB hexaploid. This hexaploid was backcrossed with G. hirsutum to produce a pentaploid. Trait selection and recurrent backcrossing to G. hirsutum resulted in plants with a restored tetraploid chromosome number and rust resistance.

Gossypium Pigment Gland Expression

All species of Gossypium, with the exception of those endemic to Australia, are normally glanded both in the seed and throughout the vegetative parts. It has been established that the glanded condition of G. hirsutum vegetation is determined by three major genes, namely $G1_1$, $G1_2$, and $G1_3$ (25). The $G1_1$ locus controls the gland condition in the hypocotyl, stems, petioles, and bolls (31) while $G1_2$ and $G1_3$ determine the gland condition in the cotyledons and leaves (32). McMichael (32) selected mutants of G. hirsutum which were completely glandless in the seed and all vegetative parts. The glandless condition is caused by a homozygous recessive condition symbolized by $gl_2gl_2gl_3gl_3$ (32). The $G1_1$ designation is not included because its expression is dependent on interaction with at least one dominant allele at either the $G1_2$ or $G1_3$ locus (32). Lee (25) concluded from interspecific hybridization studies that the $G1_2$ locus

resides in the A genome, and the $G1_3$ locus resides in the D genome. According to Rhyne (43) the $G1_1$ locus resides in the D genome.

In addition to the main gland determining genes $G1_1$, $G1_2$, and $G1_3$, other minor gland genes have been reported. Barrow and Davis (4) observed a gene allelic to $G1_2$ and designated it as $G1_2^S$. This gene in the homozygous condition $G1_2^S G1_2^S$ imparts reduced gland density in the cotyledons as compared to $G1_2$ or $G1_3$. Lee (24) found evidence for two additional leaf gland loci and designated them $G1_4$ and $G1_5$. Murray (39) reported a second locus for glanded stem and named it $G1_6$. He concluded that $G1_6$ must be a relic homologue of $G1_1$ carried on the A genome and that it has been almost completely lost from the tetraploid species. The presence of these additional loci have not been confirmed by other investigators.

Lee (25) found that in G. hirsutum $G1_2$ has approximately twice the expressivity of $G1_3$. For example the $G1_2 G1_2 g1_3 g1_3$ genotype produces more vegetative gland expression than the $g1_2 g1_2 G1_3 G1_3$ genotype. Lee (24) also found evidence to suggest that gland development on the cotyledons and true leaves proceeds stepwise from the margins inward.

The Gossypium species endemic to Australia, including G. sturtianum, contain glands on all parts of the plant except the seed and roots (15). To date the genetics of seed gland expression or lack of seed gland expression in Gossypium has not been studied other than to determine that in the AD genome the $g1_2 g1_2 g1_3 g1_3$ genotype results in glandless vegetation along with glandless seed. It is also important to note that the genetics of seed gland suppression

and vegetative gland expression have not been examined in the C genome.



CHAPTER III

MATERIALS AND METHODS

Gland Expression in Gossypium Hexaploid Hybrids

The hybrids evaluated in this study were hexaploids produced from interspecific crosses between G. sturtianum and cultivated Gossypium varieties. Gossypium sturtianum, the original male parent of the hexaploids, is a wild, perennial, lintless cotton. The seed are glandless or exhibit very few tiny glands along the margins of the cotyledons and the hypocotyl (15). The cultivated parent in each case was glanded. The original hybrids and subsequent selections based on yield and fiber quality (37, 38) were made by Dr. H. Muramoto of the University of Arizona, Tucson. No selection pressure for glandless seed had been placed on the hybrid populations. Approximately 200 seeds from each of six selections, as indicated in Figure 1, were obtained from Dr. Muramoto.

To determine the gland expression and the extent of variability in the parent seed stocks, seed gland expression in G. sturtianum and time of gland appearance in G. australe and G. hirsutum, 'Acala 44,' were examined. Gossypium australe was used to study time of seed gland appearance since seeds of G. sturtianum were not available in the numbers needed to study germination. The two are very similar inasmuch as their seeds are glandless and they both contain the C genome (15). Ten seeds of G. sturtianum and 10 seeds of G. australe were peeled and embryos examined. An additional 20 G. australe seeds

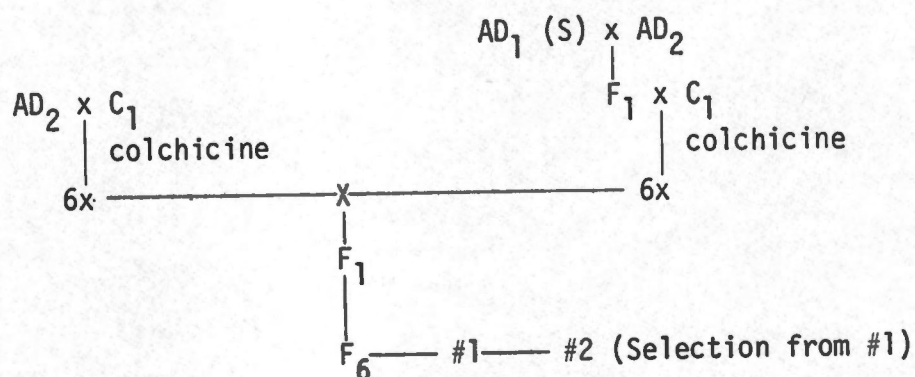
AD₁ (S) - G. hirsutum, 'Superoakra'

AD₁ (A) - G. hirsutum, 'Acala 44'

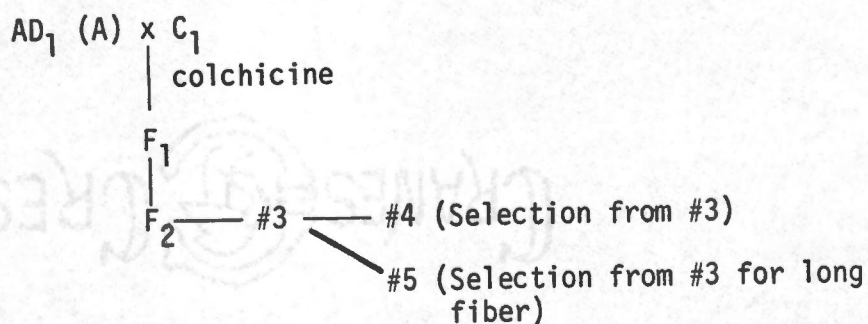
AD₂ - G. barbadense, 'PS-4'

C₁ - G. sturtianum

#1, 2



#3, 4, 5



#6

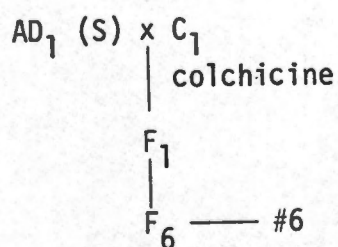


Figure 1. Hexaploid hybrid pedigrees.

were observed during the germination process. 'Acala 44,' a normally glanded commercial variety of G. hirsutum, served as the female parent for three of the original hexaploid hybrid lots under study. Approximately 2000 'Acala 44' seeds were peeled and examined for variation in gland number.

In the fall of 1979 the hexaploid hybrid seeds were screened for differences in embryo gland density. The seeds were soaked in water for 16 to 24 hours and the seed coat was removed by hand to expose the embryo. During this process it was found that some seeds had hard coats. In order to remove the hull of these seeds it was necessary to scarify the coat and immerse them in water for an additional 16 to 24 hours. After removing the seed coat the naked embryos were examined under a dissecting microscope to determine the degree of gland formation.

In the process of examining embryos it was found that gland density ranged from no glands, a rare event, to glands throughout the cotyledons and hypocotyl. Four gland categories were established: no glands (ng), very few glands (vfg), few glands (fg), and glanded (gld). The ng category contained embryos that were completely glandless. The vfg category had widely scattered glands along the margins of the cotyledons. The fg category had glands distributed along the margins of the cotyledons with some, usually lighter, glands starting to form inward from the margins. Seeds in the gld category had dark glands generally distributed throughout the cotyledons. Following the establishment of categories, each embryo subsequently screened was assigned to one of the above categories.

There were only three ng embryos and three vfg embryos among the approximately 1200 seeds examined. Two of the ng embryos did not survive. Sixty-four of the fg embryos and 47 of the gld embryos that had been examined and categorized were selected for further study. All of these embryos were germinated in a mixture of sand, soil, and vermiculite in the laboratory and then transplanted to pots and maintained in the greenhouse during the winter months. The seedlings were supplied with a complete liquid fertilizer (Miracle Grow) every two weeks.

In May 1980, 110 of these seedlings from the fg and gld seed categories, plus the three seedlings from the vfg category, were transplanted to a field plot on The University of Tennessee Plant Sciences Research Farm near Knoxville, Tennessee. The plot was on an Etowah silt loam soil with a pH of 6.7 and tested high in available P and K. Prior to planting 27, 23, and 45 kg/ha of N, P, and K, respectively, were applied broadcast. Standard seedbed preparation was followed and the seedlings were spaced approximately 90 cm apart. Water was supplied at transplanting and throughout the season by sprinkler irrigation as needed. A recommended pest control program for cotton was followed. The surviving plant derived from a glandless embryo was kept in the greenhouse.

From early July through mid-August 1980, controlled self and cross pollinations were made within each of the six lots. Plants were examined in the evening for buds which would bloom the following day. The buds to be self pollinated were enclosed in a 7 x 9.5 cm glassine envelope and secured with a paper clip. A 200 ppm solution of silver

nitrate was sprayed on the base of the peduncle to control abscission. For the buds to be cross pollinated, the petals and immature staminal column were removed with tweezers and treated as above with the addition of silver nitrate to the wound that resulted from emasculation. Hand cross pollinations were made the day following emasculation with mature pollen from another plant in the same lot.

Three times during the season a cm^2 sample was taken from the base of the first mature leaf below the apical meristem. Each sample was examined under a dissecting microscope and the number of glands recorded. As a check for leaf gland number, one leaf gland sample was taken from each of 10 G. hirsutum 'Hancock' plants and five leaf gland samples were taken from each of two G. sturtianum plants. The gland number was recorded as above.

Once during the season a cm^2 sample of a bract, approximately 3.5 cm wide, was removed and the number of glands was examined and recorded. A stem sample was taken from an area 2 cm in length between the second and third node from the apex of each plant. The stem sample was evenly split and a gland count was taken on one of the halves.

Following harvest in November, the three vfg plants plus two from the fg category and two from the sg category were moved to the greenhouse. Controlled self pollinations were made on these plants and the plant from the ng category.

The seeds harvested from the 1980 plot were screened as in 1979. They were placed in the established categories of ng, vfg, fg, and gld. Only one embryo was obtained in the ng category and

12 embryos were obtained in the vfg category. Forty-two of the fg embryos and 56 of the gld embryos were selected for further study. All of these embryos were germinated and maintained in the greenhouse as in the previous year.

In May of 1981, this second generation of seedlings was transplanted to The University of Tennessee Plant Sciences Research Farm in a plot of the same soil type, and with the same seedbed preparation, fertilization, irrigation, and pest management as in 1980. Controlled self and cross pollinations were made, and vegetative samples were taken as in 1980 with the exception that 200 ppm gibberellic acid was used instead of silver nitrate on the base of the peduncle and on emasculation wounds.

Seeds from the 1981 harvest were screened as in 1979 and 1980 with the exception of the establishment of a new category, several glands (sg). These seeds had glands generally distributed throughout the cotyledons, as in the glanded category, but the glands were dark only around the margins of the cotyledons and more faint throughout the rest of the cotyledons.

The statistical analyses were computed using a Statistical Analysis System (SAS) (3) package on an IBM 370/3031 computer.

Vegetative Gland Expression in G. arboreum x G. anomalum Hybrids

'Nanking' is a cultivated variety of G. arboreum which served as the female parent in the hybrid used to examine the B genome gland expression in the presence of the A genome. 'Nanking' leaves usually have three to five pointed or rounded lobes. The leaves, stem, and

bracts are covered with a light pubescence and the leaves, stem, bracts, and boll have a uniform distribution of small glands.

Gossypium anomalum is a wild species containing the B genome and has very large vegetative glands. The leaves are usually pointed and have three to five lobes. Relative to 'Nanking,' the pubescence covering the plant is heavy. The glands, which are densely distributed throughout the leaves, stems, bracts, and bolls, are large and protruding in comparison to those of G. arboreum.

Using the in ovulo embryo culture method described by Stewart and Hsu (48), 16 hybrid seedlings were obtained from a primary cross of G. arboreum x G. anomalum. The seedlings were transplanted into vermiculite and kept in an incubator at 30°C in 16 hours photoperiod for several days and then transplanted into a mixture of sand, soil, and vermiculite. They were maintained in the greenhouse and given a complete liquid fertilizer (Miracle Grow) every two weeks.

Upon maturity controlled self and cross pollinations among the 16 F₁ hybrids were accomplished as described above for hexaploid hybrids, except only gibberellic acid was used. The F₂ seeds were potted and maintained in the greenhouse as above. Visual observations were made on the F₂ plants including vegetative gland size and density, stem pubescence, and leaf morphology. Leaf morphology was recorded by making photocopies of representative leaves from each plant. Leaf gland size and density of G. arboreum, G. anomalum, F₁ and F₂ progeny were recorded photographically through a dissecting microscope, and leaf gland density was measured from a cm² sample taken from the

base of mature leaves. Each sample was examined under a dissecting microscope and the number of glands was recorded.



CHAPTER IV

RESULTS AND DISCUSSION

Gland Expression in Gossypium Hexaploid Hybrids

Experimental Controls

Five of the 10 ungerminated seeds of G. sturtianum were glandless and five had very few small faint glands near the margin of the cotyledons and on the hypocotyl. All the ungerminated seeds of G. australe were glandless. Ten G. australe seeds were germinated in continuous light and 10 were germinated in constant darkness at 30°C. Gland formation was observed to be part of the germination process. Both sets of seedlings exhibited glands on the elongating hypocotyl and expanding cotyledons. The approximately 2000 'Acala 44' seeds were uniformly heavily glanded with very little variation in the glanded condition among seeds.

Hexaploid Seed Gland Expression

There was a wide and continuous range in the number of seed glands in the hexaploid hybrids. However, in all cases the hybrid seeds had a reduced gland number relative to the main commercial line used as the original female parent, 'Acala 44.' It is possible that the variation was due to the presence of $G1_2^S$, gl_2 , and/or gl_3 alleles which diminish cotyledon gland expression in G. hirsutum (4, 32). However, this is probably not the case since the phenotypic cotyledon gland reduction caused by $G1_2^S$, gl_2 , and gl_3 is very distinct while

the hexaploid seed gland expression changed upon germination of the seed. More likely, the suppression of the fully glanded expression was the result of the influence of the C genome in the hybrid.

Progeny Yields

Lack of fertility was a major problem in dealing with the hexaploid plants. The addition of 200 ppm of silver nitrate in controlled self and cross pollinations in 1980 appears to have been of little or no value. Of the 455 controlled pollinations that were made, only five bolls that were self pollinated and four bolls that were cross pollinated set seeds. The addition of 200 ppm of gibberellic acid in controlled self and cross pollinations in 1981 appears to have been no better than silver nitrate. Although many treated bolls remained on the plant, most contained no seeds. Of the 221 controlled pollinations made in 1981, only nine bolls that had been self pollinated bore seeds.

There were 113 plants in the field in 1980 and only 25 bore seeds. A total of 282 mature seeds were harvested of which 236 seeds were the result of open pollinations. Nine successful controlled pollinations yielded 46 seeds. Ninety-five immature seeds were discarded because they could not be accurately categorized according to gland number. Results of the 1981 harvest were very similar to those in 1980. Of 111 plants in the field, only 18 bore a total of 126 mature seeds. Open pollinations resulted in 85 seeds while nine successful controlled self pollinations yielded 41 mature seeds. Sixty-five immature seeds were discarded.

Pollinations made on plants in a greenhouse environment were productive. The seven plants transplanted from the field to the greenhouse in 1980 yielded 602 mature seeds. Of these 366 were from open pollinations and 236 were the result of self pollinations. Seventy-eight seeds were discarded as immature. The seeds resulting from open pollination in the greenhouse were considered to be from self pollinated flowers since most seed set took place during the winter months and there was very little chance for cross pollination from insects.

Leaf Gland Expression

The leaf gland samples taken from G. hirsutum, 'Hancock,' under the same field conditions as hybrid samples, ranged from 65 to 134 glands per cm^2 . The samples taken from G. sturtianum leaves ranged from 124 to 315 glands per cm^2 . The hexaploid leaf gland count ranged from 30 to 195 glands per cm^2 in 1980 and 46 to 184 glands per cm^2 in 1981. These data indicate that G. sturtianum is more heavily glanded than G. hirsutum, 'Hancock,' and that the leaf gland density in the hybrid is intermediate between the two.

The No Gland Category

During the course of this study approximately 1600 mature hexaploid hybrid seeds were dehulled and examined. Only three seeds screened in 1979 and one seed screened in 1980 were found to be glandless. These embryos were small and weighed 60% less than the average from all other categories. Two of the embryos screened in 1979 died shortly after germination. The two surviving embryos grew

to mature plants. The first was maintained in the greenhouse and the second was transplanted to the field during the 1981 growing season. These two plants produced a total of 16 seeds, all of which had glands. Since the glandless condition appears to have been the result of underdeveloped seed, the no gland category was omitted from statistical tests and analyses.

Statistical Analyses

A Pearson's correlation was computed for hard and soft seed coat with seed gland categories and the various vegetative gland parameters. No significant correlations were obtained.

In order to determine if there was an association between the seed gland categories and the vegetative gland density, class intervals were established for the gland density of leaves, bracts, and stems. Gland density on each of these structures varied widely and there were no natural breaks or groupings in the range of values. Therefore, each class interval was established as one standard deviation of the mean gland number for each of the three structures.

Statistical tests were performed after combining the six hexaploid hybrid seed lots. This was done after examination among lots revealed few differences in the proportion of seeds in each category and the number of glands on vegetative parts. It is important to note that even after combining lots the table for testing each association was so sparse that chi-square may not have been a valid test.

A chi-square test for association between seed gland category and gland density class of each of the plant structures was computed. For

those categories showing an association, the correlation coefficient was determined in order to measure the relative importance of the association. Table 2 shows that in 1980 the seed gland category was not associated with the density of leaf glands on the mature plant. In 1981, however, there was an association between the sample 1 leaf gland density and also the average gland density with the seed gland category. Examination of the correlation coefficients revealed that the correlations were very low and therefore not biologically important.

Table 3 shows that in 1980 the seed gland category was not associated with the density of glands on the stem and bracts of the mature plant. In 1981 there was an association between the density of glands on the stem with the seed gland category. Again, the correlation coefficient was not of sufficient magnitude to be biologically important.

The chi-square test for association between the gland densities of the various vegetative structures is shown in Table 4. As expected, the three leaf samples show a highly significant association and a highly significant correlation in 1980 and 1981. Also in 1980 there was a highly significant association and correlation between the gland densities of leaf average by bract, leaf average by stem, and bract by stem. In 1981 there was a highly significant association and a significant correlation between the average leaf gland density with stem gland density, and between bract gland density and stem gland density. There was no significant association between average leaf gland density and bract gland density; however, there was a

Table 2. Chi-square test for association between seed and leaf gland density.

Leaf gland density determination	1980 Chi-square	1981 Chi-square	Correlation(r)
Sample 1	4.36	35.12*	-0.05
Sample 2	6.09	18.49	-----
Sample 3	16.41	20.88	-----
Average	7.76	40.22**	-0.08

*Indicates significance at the 5% level of probability

**Indicates significance at the 1% level of probability

Table 3. Chi-square test for association between gland density in seeds and in bracts and stems.

	1980 Chi-square	1981 Chi-square	Correlation(r)
Bract gland density	8.24	12.57	-----
Stem gland density	10.76	37.92*	0.18*

*Indicates significance at the 5% level of probability

Table 4. Chi-square test for association between vegetative gland parameters.

	1980		1981	
	Chi-square	Correlation(r)	Chi-square	Correlation(r)
Leaf gland sample 1 by leaf gland sample 2	64.82**	0.56**	81.68**	0.68**
Leaf gland sample 1 by leaf gland sample 3	68.91**	0.56**	77.48**	0.56**
Leaf gland sample 2 by leaf gland sample 3	186.85**	0.58**	168.44**	0.80**
Leaf gland density average by bract gland density	142.56**	0.37**	16.05	0.32**
Leaf gland density average by stem gland density	67.40**	0.43**	67.90**	0.19*
Bract gland density by stem gland density	72.62**	0.40**	74.89**	0.26*

*Indicates significance at the 5% level of probability

**Indicates significance at the 1% level of probability

highly significant correlation between the average leaf gland density and the bract gland density. With the latter three correlations in 1981 the coefficients were low and not of sufficient magnitude to be biologically important.

In designing this study it was realized that the gland densities in the various vegetative structures would not necessarily be genetically linked, hence the reason gland measurements were taken from bracts and stems as well as from leaves. The low vegetative gland correlations were probably due to the independent actions of $G1_1$, which control the gland condition in the hypocotyl, stems, petioles, and bolls of G. hirsutum (31) and $G1_2$ and $G1_3$ which determine the gland condition in the cotyledons and leaves of G. hirsutum (32). Also, although the gland determining gene(s) in G. sturtianum have not been examined, it is possible that they contributed to the variation.

An analysis of variance presented in Tables 5 and 6 was performed on the 1980 and 1981 field data with the seed gland category serving as the independent variable. In both years there was no significant variation in leaf gland samples 1, 2, 3, average leaf gland density, bract, and stem gland densities due to seed category.

Table 7 shows the results of statistical tests used to determine if vegetative gland densities are normally distributed within the fg and gld seed categories for 1980 and 1981. In 1980 the values in each of the three leaf gland samples as well as the average leaf gland densities and the bract gland densities within both the fg and gld categories were found to be normally distributed. Also, the stem

Table 5. Analysis of variance for leaf gland density determination.

Source of variation	Sample 1	Sample 2	Sample 3	Average
<u>1980</u>				
Seed gland category	37 (MS)	1132 (MS)	1129 (MS)	284 (MS)
Error	1069	582	716	561
<u>1981</u>				
Seed gland category	9619 (MS)	2112 (MS)	2579 (MS)	3283 (MS)
Error	4247	3795	2995	2871

Table 6. Analysis of variance for gland density in bracts and stems.

Source of variation	Bract gland density	Stem gland density
<u>1980</u>		
Seed gland category	172 (MS)	5684 (MS)
Error	154	3409
<u>1981</u>		
Seed gland category	197 (MS)	7922 (MS)
Error	134	3453

Table 7. Statistical tests to determine if vegetative gland densities within a category differ from a normal distribution.

	Values from normality tests					
	1980			1981		
	Few gland category	Skewness	Glanded category	Skewness	Few gland category	Glanded category
Leaf gland density determination						
Sample 1	D 0.08	-----	W 0.97	-----	D 0.16**	D 0.18**
Sample 2	D 0.09	-----	W 0.98	-----	D 0.18**	D 0.08
Sample 3	D 0.10	-----	W 0.98	-----	D 0.18**	D 0.06
Average	D 0.09	-----	W 0.96	-----	D 0.17**	D 0.18**
Bract gland density	D 0.08	-----	W 0.97	-----	D 0.86**	D 0.08
Stem gland density	D 0.12**	0.94**	W 0.97	-----	-----	D 0.12**
1.98**						

1.96**						

0.71**						
Shapiro-Wilk (W) Statistic computed in tests where number of observations < 50						
Kolmogorov-Smirnov (D) Statistic computed in tests where number of observations > 50						
**Indicates significance at the 1% level of probability						

gland density in the gld seed category was found to be normally distributed. The stem gland density in the fg category exhibited a bimodal distribution and was skewed by four large outlying observations from a total of 64 observations.

The normal distribution tests for 1981 revealed that four of the 12 distributions were normal. The remaining eight distributions were skewed. Again, as in 1980, the skewness was usually caused by a small number of observations that had values much larger than the main body of observations. The large values were probably sampling errors incurred while counting the gland numbers on vegetative samples.

Table 8 shows the average gland density on vegetative parts of plants and the standard deviation of the observations from the fg and gld seed categories in 1980 and 1981. It is important to note that there was as much or more variation in vegetative gland density within categories as between categories.

The above data are evidence that gland expression in the seed is independent of the subsequent vegetative gland density. The data indicate that the two conditions are inherited separately, therefore it should be possible to select for low seed gland expression while maintaining or increasing vegetative gland density.

Seed Gland Category Observations

The original seeds and the seeds from the 1980 field harvest were categorized as ng, vfg, fg, and gld. Based on these results a fifth category, sg, could appropriately be added between the fg and gld categories when greenhouse seeds and 1981 field seeds were screened. This increased the distinction between the fg and the gld categories.

Table 8. Average gland number on plants from different seed gland categories.

	Leaf		Bract		Stem	
	$\bar{X}$	$S\bar{x}$	$\bar{X}$	$S\bar{x}$	$\bar{X}$	$S\bar{x}$
<u>1980</u>						
Few glands	95	3.3	35	1.6	187	8.5
Glanded	92	2.9	37	1.9	170	6.4
<u>1981</u>						
Few glands	125	7.6	34	2.3	199	9.1
Glanded	117	5.9	34	1.5	210	6.7

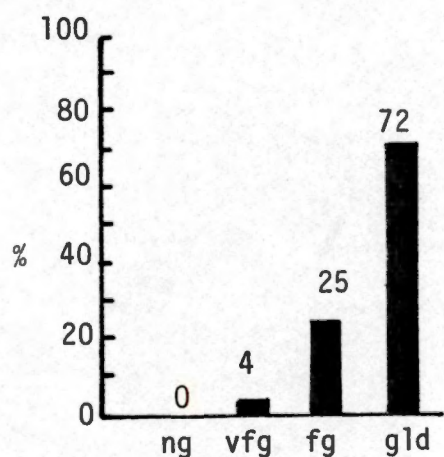
Figures 2 and 3 are standardized graphs, based on 100 seeds, of the relative numbers of seeds obtained in each category according to the category of the parent(s). As with the statistical analyses, lots were combined because of low yields and the similarity of results among individual lots. Figure 2A represents the seeds obtained from 13 open pollinated plants in the fg category that bore seeds in 1980. Figure 2B represents the progeny obtained from the 14 open pollinated plants in the gld category that bore seeds in 1980. Similarly, Figure 2C and 2D represent the six fg and seven gld open pollinated plants that bore seeds in 1981. There were too few field progeny from the vfg category in either year for the data to be meaningful.

The open pollinated field material represented in Figure 2 showed more variation between years than between seed categories. For example, both the fg and the gld female parents in 1980 yielded approximately 75% gld seeds and 20% fg seeds. However, the 1981 field material was much more variable. The fg female parents yielded approximately 70% fg seeds and 25% sg seeds. The gld female parents yielded approximately 45% fg seeds, 25% sg seeds, and 25% gld seeds. If the sg category had not been created, these seeds would have fallen about equally into the fg and gld categories.

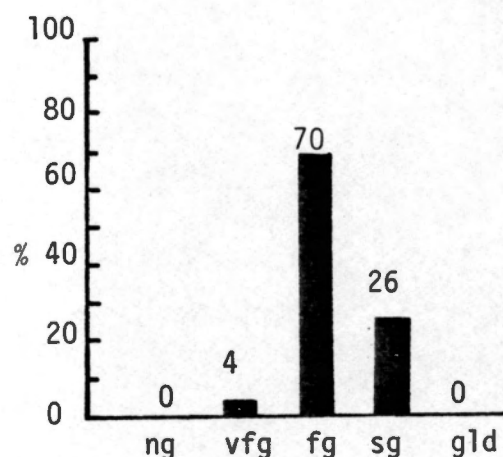
An examination of the lineage of the plants yielding seeds in each of the two years revealed no obvious genetic explanation for the variation. The observed variations were probably due to environmental differences between the two years.

The progeny from self pollinated vfg, fg, and gld parents in 1980 and 1981 and greenhouse progenies from selfed and open pollinated

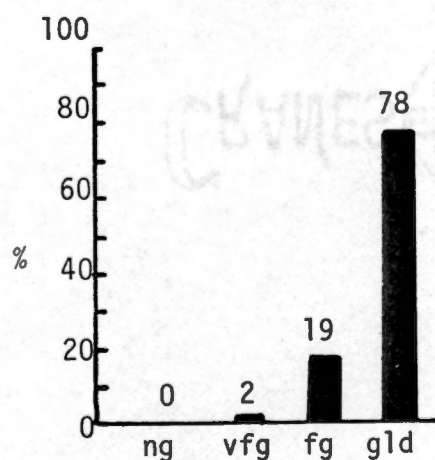
A. 1980 13 fg plants produced
101 seeds



C. 1981 6 fg plants produced
23 seeds



B. 1980 14 gld plants produced
144 seeds



D. 1981 7 gld plants produced
37 seeds

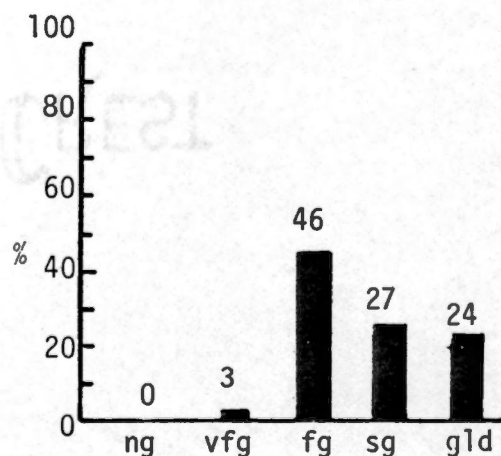
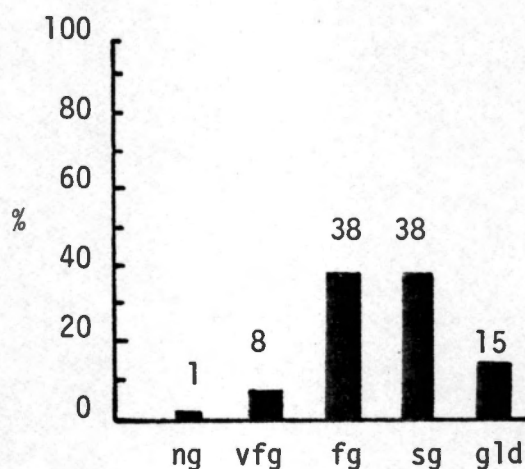
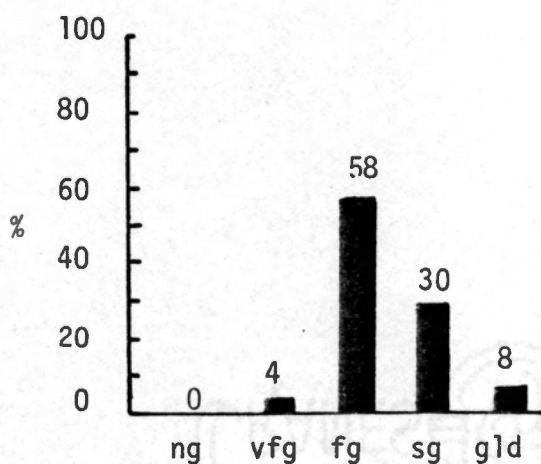


Figure 2. Relative distribution of gland density among seeds produced in 1980 and 1981 on open pollinated, field grown few glanded (fg) and glanded (gld) advanced generation plants of a hexaploid hybrid cotton, $2(AD_x \times C_1)$.

A. 1981 2 vfg plants produced
123 seeds



B. 1981 4 fg plants produced
310 seeds



C. 1981 6 gld plants produced
232 seeds

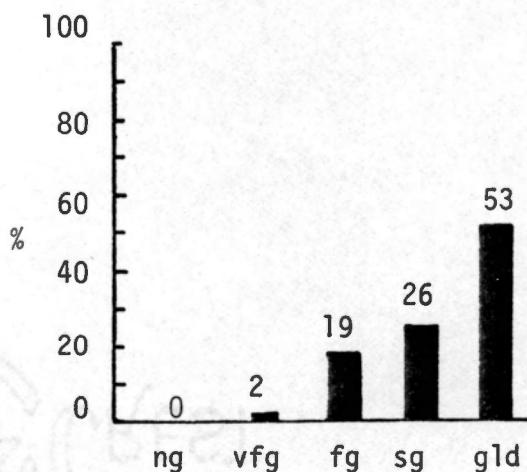


Figure 3. Relative distribution of gland density among seeds produced in 1981 on self pollinated field and greenhouse grown very few glanded (vfg), few glanded (fg), and glanded (gld) advanced generation plants of a hexaploid cotton hybrid, $2(AD_x \times C_1)$.

plants are represented in Figure 3A, B, and C. As previously mentioned, the greenhouse open pollinated material was considered to be selfed because most bolls were set during the winter and insect activity was low throughout the year.

The progenies of the plants in Figure 3A, B, and C give evidence that a given seed category yields a high percentage of like progeny. The self pollinated fg category plants yielded 58% fg seeds, 30% sg seeds, and only 8% gld seeds. The self pollinated gld category plants yielded 19% fg seeds, 26% sg seeds, and 53% gld seeds. This suggests that selection pressure for low seed gland density can be placed on a population based on seed gland categories.

The self pollinated vfg category plants did not yield as many like progeny as did the fg and gld categories that were self pollinated. Figure 3A shows that the plants from vfg seed bore only 8% vfg seeds, 38% fg seeds, 38% sg seeds, and 15% gld seeds. This may indicate that for each succeeding generation of selection for reduced seed glands, progress would move more slowly as the glandless condition is approached. However, it should be pointed out that only two vfg plants yielded the total 123 seeds. Thus, the genetic base is quite narrow and may not be a true representation of the population.

Results of the observations that have been made on the hexaploid seeds suggest that seed gland density inheritance is quantitative. The seed gland density range, from vfg to gld, is characteristic of quantitative inheritance. Also the wide range in gland density of the progeny from individual parent categories is characteristic of quantitative inheritance.



A Delayed Gland Theory

The following theory is projected to explain the variation in seed glands. As mentioned earlier, the cotyledon gland expression in the AD genome, including G. hirsutum and G. barbadense, involves the two major genes called Gl_2 and Gl_3 . Also, reduced gland alleles such as Gl_2^S (4) may influence the gland condition of the cotyledons. The gene(s) responsible for the glandless and near glandless seed trait of G. australe and G. sturtianum in the C genome have not been elucidated.

Results of observations on the glandless and near glandless seeds and on the appearance of glands during the germination process indicate that the gene(s) controlling the C genome seed trait are regulatory rather than structural. They do not prevent the formation of glands, but rather control the time at which terpenes form in the gland structures. The rapid appearance of glands in conjunction with germination indicates that the gland sites are pre-established and that terpene synthesis is the factor that is delayed until germination.

As outlined in Figure 4, Gl_2 and Gl_3 are the major genes responsible for the glanded condition in the seeds and vegetative parts of plants in the AD genome. For the C genome it is assumed that there is one major vegetative gland gene and it is here designated as Gl_C . The theoretical "delayed gland" gene(s) is designated as Dg. The glanded vegetative plant parts in the C genome would be due to the presence of the Gl_C gene(s) and the glandless seeds would be due to

$G1_2G1_2G1_3G1_3 \longrightarrow \text{Glanded Seed } AD_1$
 $(G1_CG1_C) + (DgDg) \longrightarrow \text{Glandless Seed } C_1$

Figure 4. Action of the major gland determining genes in G. hirsutum and G. barbadense, and proposed gland determining genes in G. sturtianum.

the action of the Dg gene(s) which delays gland formation until germination.

The scheme for the reduced gland condition in the hexaploid seeds, as indicated in Figure 5, is as follows. In the hexaploid condition all the genes of the AD genome, from G. hirsutum and/or G. barbadense, and the C genome, from G. sturtianum, are combined. Gl₂ and Gl₃ genes from the AD genome and Gl_C from the C genome would act to produce glands in the vegetative parts of the plant. That would leave chiefly the Dg gland gene(s) from the C genome to interact and regulate the gland condition of the hexaploid seeds. It is important to note that the differences observed in the hybrid seed glands could have been due to variation, such as Gl₂^S type alleles, in the AD genome. This is not likely because of the continuous range of seed gland density in the seeds and the fact that approximately 2000 G. hirsutum seeds, 'Acala 44,' exhibited a uniform gland condition. Therefore the reduced gland condition and variation in gland density of the ADC hexaploid seeds is probably due to the effects of a Dg type gene(s) in the C genome.

It is possible that a portion of the variation in the glanded condition of the hybrid seeds was due to intergenomic genetic exchange. Intergenomic pairing and/or exchange between A and C or D and C chromosomes containing genes determining the time of gland appearance could result in an increased effect from one of the genomes. However, extensive recombination between genomes is not likely since cytological examination of the AADDCC hexaploids revealed very little intergenomic pairing (10).

$$Gl_2Gl_2Gl_3Gl_3(Gl_cGl_c) + (DgDg) \longrightarrow \text{Reduced glands } AD_1C_1$$

Figure 5. Proposed interaction of the AD and C genomes in determining gland expression.

The possibility of a "delayed gland" gene(s) in the C genome which can function phenotypically in the presence of AD gland alleles offers promising possibilities for transfer into agronomic lines of the AD genome. As outlined earlier, the glandless seed trait would be of much value in commercial cotton seed.

Vegetative Gland Expression in G. arboreum x G. anomalum Hybrids

The F₁ Plants

The F₁ hybrids were very uniform in morphology. The leaves had three to five rounded or pointed lobes as compared to G. arboreum and G. anomalum in Figure 6. All aerial parts of the plants were covered with a downy pubescence and a uniform distribution of glands that varied in size. These plants bloomed prolifically at maturity and approximately 880 self and cross pollinations were performed. Since the A₂ and B₁ genomes are relatively close, in homoeology, some fertility was observed in the F₁ diploid hybrids.

The F₂ Progeny

A total of 28 viable seeds were obtained from the F₁ plants. Three seeds were the result of self pollinations performed on two plants. The other 25 seeds were the result of open pollinations from all of the F₁ plants.

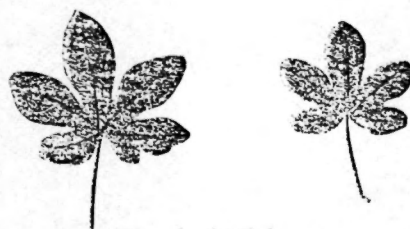
The F₂ plants exhibited a wide range of variation in stem pubescence, gland size and density, bract morphology, and leaf morphology. Leaf morphology variations, as shown in Figure 7, were very striking among individual plants. Leaf shapes ranged from five



G. arboreum
cv. 'Nanking'



G. anomalum



F₁ hybrids

Figure 6. Leaf morphology of Gossypium arboreum, Gossypium anomalum, and their F₁ hybrids. 1/8 actual size.

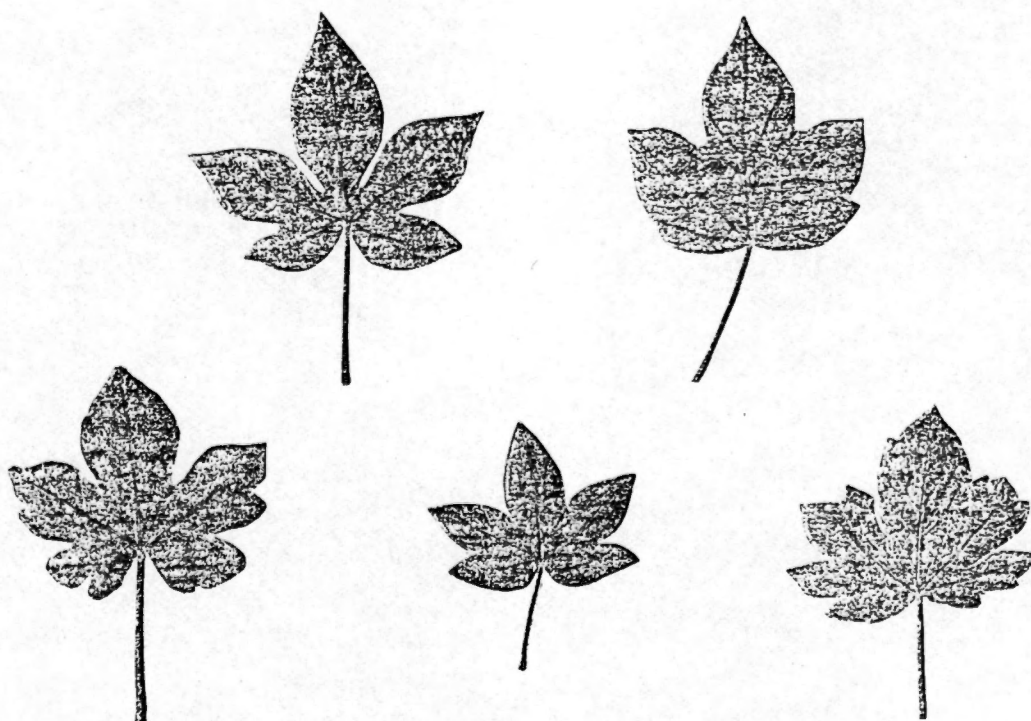


Figure 7. Leaf morphology of representative F₂ plants of a cross Gossypium arboreum x Gossypium anomalum. 1/8 actual size.

deeply cut pointed lobes to three medium cut lobes. Some had ruffled margins and overlapping lobes.

Observations on leaf gland density yielded evidence of quantitative inheritance. The gland number was counted from cm^2 samples taken from the base of two mature leaves of each F_2 plant, from two F_1 hybrids, and from two each of the original parents, G. arboreum (A_2), 'Nanking,' and G. anomalum (B_1). The G. arboreum samples averaged 39 glands per cm^2 , the G. anomalum samples averages 134 glands per cm^2 , and the F_1 hybrid averaged 82 glands per cm^2 . The F_2 hybrid average gland density ranged from 46 to 184 per cm^2 . The gland density per cm^2 of A_2 , B_1 , the F_1 hybrids, and the 28 F_2 hybrids was ranked by gland density and graphed in Figure 8. The distribution is a very good example of quantitative inheritance. The F_1 gland density falls approximately midway between the parent species, and the F_2 gland densities fall along and exceed the range of the A_2 and B_1 species. The F_2 plants with gland densities above that of the B_1 species may be exhibiting heterosis.

Photographs of a portion of the base of a mature leaf of representative samples, magnified 4.5 times, are shown in Figure 9. Included is a sample of G. arboreum, G. anomalum, an F_1 hybrid, and six F_2 plants, numbered 1 through 6. Sample 1 is from an F_2 plant that resembles G. arboreum and sample 3 is from an F_2 plant that resembles G. anomalum. Sample 2 is from an F_2 plant that resulted from the successful self pollination of an F_1 flower and resembles the F_1 sample. Samples 4 through 6 are from F_2 plants that outperformed G. anomalum in average sample gland density. It is important

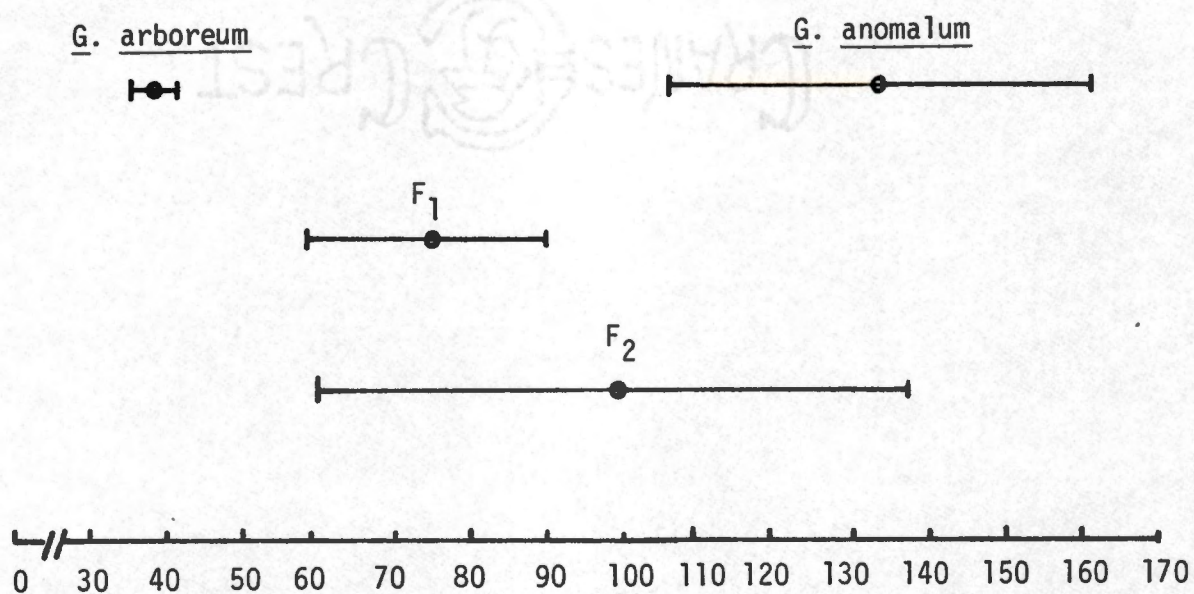


Figure 8. Mean and standard deviation of glands per cm^2 of leaf area on: 2 G. arboreum plants, 2 G. anomalum plants, 4 F_1 hybrid plants, 28 F_2 hybrid plants.

CHINESE-AMERICAN

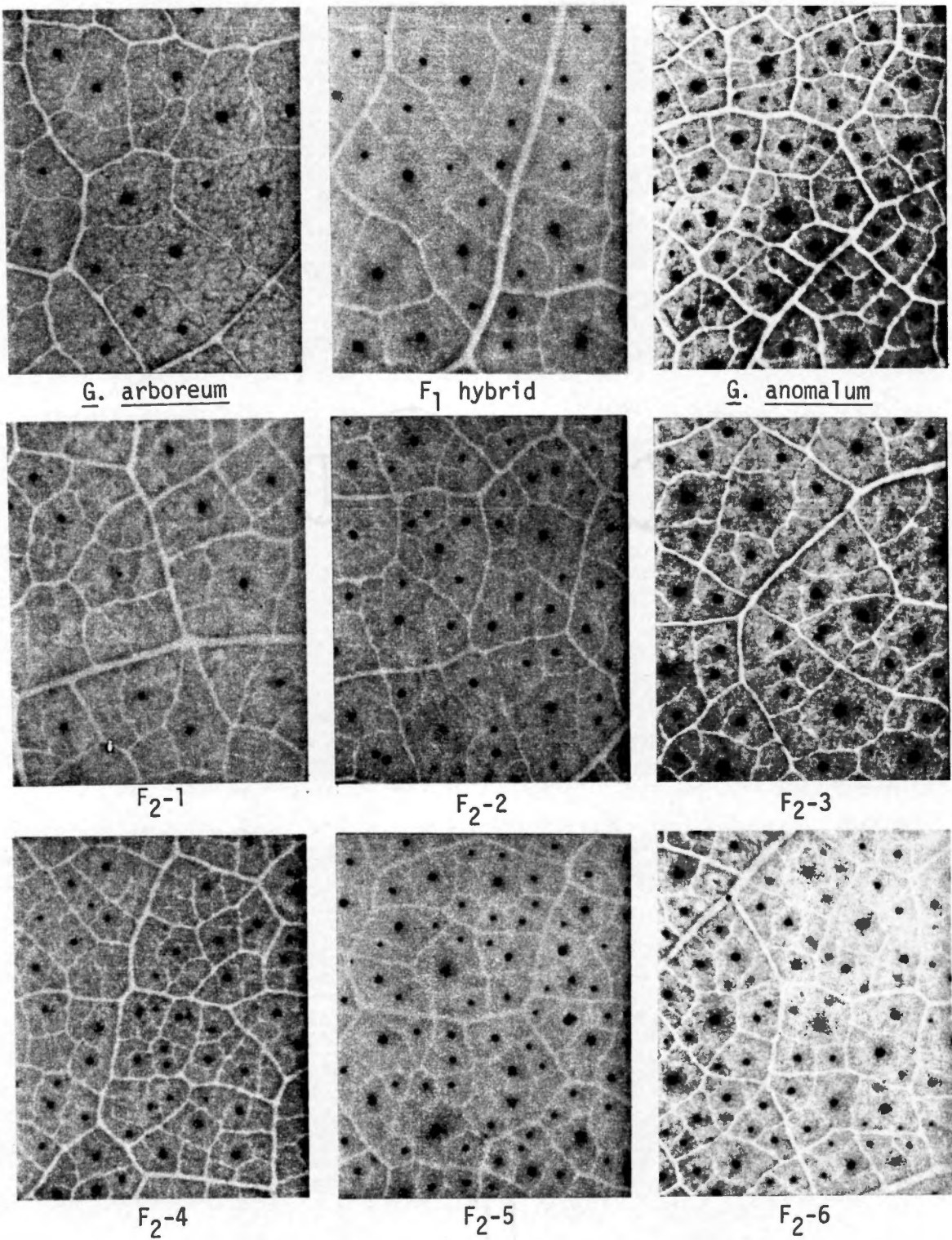


Figure 9. Photographs of a portion of the base of a mature leaf of G. arboreum, G. anomalum, G. arboreum x G. anomalum F_1 hybrid, and six F_2 samples. 14 times actual size.

to recognize that sample 3 (and to some extent samples 4 through 6) exhibits small glands and also large glands that resemble those of G. anomalum. The F_2 plants with small and large glands may be expressing both genes.

If the A and B genome gland gene(s) are homoeologous it should be feasible to introgress the B_1 trait into the A_2 genome. Then an artificial hybrid (49) would be made between G. arboreum (A_2) and G. hirsutum (AD_1) for introgression of the large gland trait into the AD genome.

CHAPTER V

SUMMARY AND CONCLUSIONS

The glands found in Gossypium can be both useful and deleterious in the production and utilization of cotton. High terpene levels, characteristic of glands, are desirable for insect resistance but undesirable for seed consumption. The wild species G. sturtianum has almost glandless seed but a strongly glanded plant, and G. anomalum has large glands in the foliage, especially on the carpel walls. This study is a beginning examination of the genetics and possible transfer of specific gland traits from these two wild species of Gossypium to commercial cotton.

In hexaploid hybrids involving G. sturtianum and cultivated cotton, the C genome suppressed a portion of the seed gland expression of the AD genome. This is indicated by the seed gland expression which ranged from a glanded condition that was less than the check variety of G. hirsutum, to a few faintly developed glands on the margins of the cotyledons and hypocotyl, a condition that approached the gland expression in G. sturtianum seeds. Particularly important was the strong evidence that vegetative gland inheritance is independent of seed gland inheritance in the hexaploid. Seed gland expression appears to be quantitatively inherited. Building from the evidence of these data one should be able to isolate a population of hexaploid seeds with a very low seed gland density and a high complement of glands on the resultant vegetative parts. This population would then

be backcrossed with glandless G. hirsutum for trait selection and introgression into commercial cottons.

Data from G. arboreum x G. anomalum hybrids yielded evidence that the large gland trait of G. anomalum is quantitatively inherited. With a degree of fertility in hybrids of $A_2 \times B_1$ it should be possible to isolate the trait into an A_2 background and then hybridize G. arboreum (A_2) with G. hirsutum (AD_1) for introgression into the A genome of commercial cottons. Since the A_2 and the A genome of G. hirsutum have a high level of homoeology, the trait can be transferred to commercial cottons by recurrent selection from backcrosses of $2(A_2AD_1)$ to AD_1 .



LITERATURE CITED

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1. Abou-Donia, M. B. 1976. Physiological effects and metabolism of gossypol. *Residue Rev.* 61:125-160.
2. Adams, R., and T. A. Geissman. 1960. Gossypol, a pigment of cottonseed. *Chem. Rev.* 60:555-574.
3. Barr, A. J., W. H. Blair, D. M. Chilko, J. H. Goodnight, and J. P. Sall. 1979. *SAS User's Guide*. SAS Institute Inc. Cary, North Carolina.
4. Barrow, J. R., and D. D. Davis. 1974. $G1_2^S$ --A new allele for pigment glands in cotton. *Crop Sci.* 14:325-326.
5. Beasley, J. O. 1942. Meiotic chromosome behavior in species, species hybrids, haploids, and induced polyhaploids of Gossypium. *Genetics* 27:25-54.
6. Berardi, L. C., and L. A. Goldblatt. 1980. Gossypol. p. 183-237. In I. E. Liener (ed.) *Constituents of Plant Foodstuffs*. Academic Press, New York.
7. Bottger, G. T., E. T. Sheehan, and M. J. Lukefahr. 1964. Relation of gossypol content of cotton plants to insect resistance. *J. Econ. Entomol.* 57:283-285.
8. Carter, C. M. 1972. Cottonseed protein: Its current status and future prospects as a human food. p. 102. In *Proc. Beltwide Cotton Prod. Research Conf. Memphis, Tennessee. 10-12 Jan. 1972.* National Cotton Council, Memphis, Tennessee.
9. Carter, F. L., A. E. Castillo, V. L. Frampton, and T. Kerr. 1966. Chemical composition studies of seeds of the genus Gossypium. *Phytochem.* 5:1103-1112.
10. da Silva, F. P., H. Muramoto, J. E. Endrizzi, and L. S. Stith. 1975. Chromosome behavior in the hexaploid cotton Gossypium hirsutum L. x Gossypium sturtianum Willis. p. 87. In *Proc. Beltwide Research Conf. New Orleans, Louisiana. 6-8 Jan. 1975.* National Cotton Council, Memphis, Tennessee.
11. Demol, J., et R. Maréchal. 1975. Contribution à l'étude la résistance à la sécheresse de quelques cotonniers allohexaploïds. *Bull. Rech. Gembloux* 10(2):151-162.

12. Demol, J., L. Verschraege, et R. Maréchal. 1976. Utilisation des espèces sauvages en amélioration cotonnière caractéristiques technologiques des formes allohexaploïdes. *Coton Et Fibres Tropicales* 31:381-389.
13. Eagle, E. 1960. A review of some physiological effects of gossypol and cottonseed pigment glands. *J. Amer. Oil Chemists Soc.* 37:40-43.
14. Edwards, G. A., and M. A. Mirza. 1979. Genomes of the Australian wild species of cotton. II. The designation of the new G genome for Gossypium bickii. *Can. J. Genet. Cytol.* 21:367-372.
15. Fryxell, P. A. 1968. A redefinition of the tribe Gossypieae. *Bot. Gaz.* 129(4):296-308.
16. Fryxell, P. A. 1976. Germplasm utilization: Gossypium, a case history. USDA-ARS Bulletin ARS-S-137.
17. Fryxell, P. A. 1980. The natural history of the cotton tribe. Texas A&M University Press, College Station, Texas.
18. Harland, S. C. 1940. New polyploids in cotton by use of colchicine. *Trop. Agric.* 17:53-55.
19. Harms, W. S., and K. T. Holley. 1951. Hypoprothrombinemia induced by gossypol. *Proc. Soc. Expt. Biol. Med.* 77:297-299.
20. Harper, G. A., and K. J. Smith. 1968. Status of cottonseed protein. *Econ. Bot.* 22:63.
21. Jenkins, J. N. 1977. Assessment of pest management for glandless cotton: agronomic viewpoint. p. 110-117. *In Proc. Conf. on Glandless Cotton.* Dallas, Texas. 13-14 Dec. 1977. USDA-ARS.
22. Knight, R. L. 1955. The genetics of jassid resistance in cotton V. Transference of hairiness from Gossypium arboreum to G. barbadense. *J. Genet.* 53:150-153.
23. Kromer, G. W. 1977. Current status and future market potential for cottonseed. p. 1-9. *In Proc. Conf. on Glandless Cotton.* Dallas, Texas. 13-14 Dec. 1977. USDA-ARS.
24. Lee, J. A. 1962. Genetical studies concerning the distribution of pigment glands in the cotyledons and leaves of upland cotton. *Genetics* 47:131-142.
25. Lee, J. A. 1965. The genomic allocation of the principal foliar-gland loci in Gossypium hirsutum and Gossypium barbadense. *Evolution* 19:182-188.

26. Lukefahr, M. J., G. T. Bottger, and F. Maxwell. 1966. Utilization of gossypol as a source of insect resistance. p. 215-222. In Proc. Beltwide Cotton Prod. Research Conf. Memphis, Tennessee. TT-12 Jan. 1966. National Cotton Council, Memphis, Tennessee.
27. Lukefahr, M. J., and D. F. Martin. 1966. Cotton-plant pigments as a source of resistance to the bollworm and tobacco budworm. J. Econ. Entomol. 59:176-179.
28. Lusas, E. W., J. T. Lawhon, S. P. Clark, S. W. Matlock, W. W. Meinke, D. W. Mulsow, K. C. Rhee, and P. J. Wan. 1977. Potential for edible protein products from glandless cottonseed. p. 31-43. In Proc. Conf. on Glandless Cotton. Dallas, Texas. 13-14 Dec. 1977. USDA-ARS.
29. Martinez, W. H., L. C. Berardi, L. A. Goldblatt, 1971. Potential of cottonseed: Products, composition, and use. p. 248-261. In Proc. 3rd Intern. Congr. Food Sci. Technol. (505/70). Washington, D.C. 1970.
30. Maxwell, F. G., H. N. Lafever, and J. N. Jenkins. 1965. Blister beetles on glandless cotton. J. Econ. Entomol. 58:792-793.
31. McMichael, S. C. 1954. Glandless boll in upland cotton and its use in the study of natural crossing. Agron. J. 46:527-528.
32. McMichael, S. C. 1960. Combined effects of glandless genes gl_2 and gl_3 on pigment glands in the cotton plant. Agron. J. 52:385-386.
33. Meyer, J. R. 1957. Origin and inheritance of D_2 smoothness in upland cotton. J. of Heredity XLVIII(5):249-250.
34. Meyer, J. R., and V. G. Meyer. 1961. Origin and inheritance of nectariless cotton. 1961. Crop Sci. 1:167-169.
35. Meyer, V. G. 1974. Interspecific cotton breeding. Economic Botany 28:56-60.
36. Miravalle, R. J. 1968. Glandless cotton research. p. 117-119. In Proc. Beltwide Cotton Prod. Research Conf. Hot Springs, Arkansas. 9-10 Jan. 1968. National Cotton Council, Memphis, Tennessee.
37. Muramoto, H. 1969. Hexaploid cotton: Some plant and fiber properties. Crop Sci. 9:27-29.
38. Muramoto, H. 1975. Registration of Arizona 6X-3, and Arizona 6X-50 cotton germplasm. Crop Sci. 15:606.

39. Murray, J. C. 1965. A new locus for glanded stem in tetraploid cotton. *J. Hered.* 56:42-44.
40. Murray, J. C., L. M. Verhalen, and D. E. Bryan. 1965. Observations of the feeding preference of the striped blister beetle, Epicauta vitatta (Fabricius), to glanded and glandless cottons. *Crop Sci.* 5(2):189.
41. Phillips, L. L., and M. A. Strickland. 1966. The cytology of a hybrid between Gossypium hirsutum and G. longicalyx. *Can. J. Genet. Cytol.* 8:91-95.
42. Quaintance, A. L., and C. T. Bures. 1905. The cotton bollworm. *USDA Bur. Entomol. Bull.* 50.
43. Rhyne, C. L. 1962. Diploidization in G. hirsutum as indicated by glandless stem and boll inheritance. *Amer. Nat.* 96:265-276.
44. Rhyne, C. L., F. H. Smith, and P. A. Miller. 1959. The glandless leaf phenotype in cotton and its association with low gossypol content in the seed. *Agron. J.* 51:148-152.
45. Shaver, T. N., and M. J. Lukefahr. 1969. Effect of flavonoid pigments and gossypol on growth and development of the bollworm, tobacco budworm, and pink bollworm. *J. Econ. Entomol.* 62:643-646.
46. Skovsted, A. 1934. Cytological studies in cotton. II. Two interspecific hybrids between Asiatic and New World cottons. *J. Genet.* 28:407-424.
47. Smith, K. J. 1972. Cottonseed products. p. 40-43. *In* Cotton. CIBA-GEIGY, LTD. Basle, Switzerland.
48. Stewart, J. M., and C. L. Hsu. 1977. In-ovulo embryo culture and seedling development of cotton Gossypium hirsutum L. *Planta* 137:113-117.
49. Stewart, J. M., and C. L. Hsu. 1978. Hybridization of diploid and tetraploid cottons through in-ovulo embryo culture. *J. of Hered.* 69:404-408.
50. Thaung, U. K., A. Gros, and R. O. Feuge. 1961. Characterization of oils from low-gland and glandless cottonseed. *J. Amer. Oil Chemists Soc.* 38:220-224.
51. Vix, H.L.E., and M. G. Lambou. 1970. Cottonseed flour--glandless--glanded. p. 63-76. *USDA-ARS-72*.

52. Watkins, L. R. 1977. Impact of gland pigments upon traditional cottonseed processing procedures. p. 10-12. In Proc. Conf. on Glandless Cotton. Dallas, Texas. 13-14 Dec. 1977. USDA-ARS.

VITA

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