AN ALLELE FOR HIGH GLANDING AT THE Gl_3 LOCUS?

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Abstract

The high glanding trait (i.e. presence of gossypol glands on the lobes or crown of the cotton flower bud calyx) confers a significant level of resistance to bollworm and tobacco budworm. Until recently, all cultivated upland cotton cultivars were normal glanding (i.e. lacked glands in this area of the calyx). Three new cultivars, Hartz H1215, H1220, and H1244, express the high glanding trait, transferred from Socorro Island (a wild Gossypium hirsutum) via a breeding line, XG-15. In 1994, an effort was begun at the Delta Res. and Ext. Ctr. to determine the inheritance of the high glanding trait present in XG-15.

One of the parents used in the study was a monomeric line, XG-15gl_2Gl_3, developed by Dr. Joshua Lee. This line had glandless alleles (gl_2gl_2) at the Gl_2 locus, and dominant Gl_3 alleles from XG-15. The line was high glanding, which led to the hypothesis that the high glanding trait was conferred by a special Gl_3 allele derived from the Socorro Island cotton and passed on to the high glanding Hartz cultivars.

Crosses of high glanding x normal glanding parents resulted in high glanding F_1's and F_2 populations that segregated 3 high glanding:1 normal glanding; crosses of XG-15gl_2Gl_3 x normal glanding produced similar results. The cross of XG-15 gl_2Gl_3 x high glanding did not segregate for calyx crown glanding type. The cross of XG-15gl_2Gl_3 x glandless parents resulted in glandless squares in the F_1 and a ratio of 3 glandless (squares): 1 high glanding in the F_2.

Data available to date are consistent with the hypothesis of a special Gl_3 allele (tentatively identified as Gl_3s) derived from Socorro Island cotton. Based on the phenotype of XG-15gl_2Gl_3, it should be possible to develop cultivars that express the high glanding trait, but have reduced seed gossypol by using recessive alleles at the Gl_2 locus which has been identified as more potent in affecting seed gossypol.

Introduction

During the 1960's and 1970's, there was great interest in the genetics of gossypol production in cotton. On one hand, McMichael (1960) had identified alleles (gl_2 and gl_3) that effectively removed gossypol from cotton seed (as well as foliage), making it possible to use cotton seed products as feed for non-ruminants without the deleterious effects of gossypol. On the other hand, Lukefahr and Houghtaling. (1969) and others had begun to develop cotton strains with gossypol levels high enough to suppress bollworm (BW) and tobacco budworn (TBW). A tremendous amount of research was conducted to determine the effects of glanding alleles both native and exotic to Gossypium hirsutum. Bell and Stipanovic (1977) reported an exhaustive review of the genetics of gossypol production and gossypol glanding at this meeting, which I will not repeat. To simplify, the primary glanding loci in upland cotton are Gl_2 and Gl_3 (McMichael, 1960). Plants that are homozygous recessive at both loci (i.e. $gl_2gl_2gl_3gl_3$) have glandless seed and foliage. From studies with various combinations of native and exotic alleles at these loci, we know a lot about the expression of these genes in the cotton seed, seedling, leaf, stem, flower bud, sepal, petal, stamen, style, ovary, and carpel.

Many breeders and geneticists interested in increasing insect resistance by manipulating gossypol focused on quantifying gossypol content. Some researchers did address gossypol gland distribution and density. Despite detailed research on the distribution of glands on areas such as cotyledons, hypocotls and even flower and flower bud parts, there appears to be little direct reference to genetic control of gossypol glands located on the lobes of the calyx crown (an area normally free of glands in cultivated G. hirsutum), even though the presence of glands in this area was suggested as an aid in selecting for high gossypol content (Sappenfield et al., 1974).

It is glanding in precisely this area, the calyx crown, that later entomological research indicated to be the most useful in conferring resistance to BW and TBW. Parrot et al. (1983) demonstrated that early instar TBW, once they move out of the plant terminal, prefer initially to feed along the margin of the calyx crown. They later demonstrated that the presence of gossypol glands in this area is a strong deterrent and antibiotic factor against TBW (Parrot et al, 1989; Hedin et al., 1992). This finding changed the focus of some breeding programs aimed at insect resistance. Breeders could now, in theory, achieve a significant level of insect resistance, without dramatic increases in seed gossypol, by breeding for the presence of glands on the calyx crown (a characteristic I refer to as "high glanding"). This approach has been used with some success (Calhoun and Jones, 1994).

Despite success in developing high yielding strains with the high glanding trait, there is still no widely accepted understanding of how the trait is inherited. The only effort I am aware of to determine the inheritance of the high glanding trait is a dissertation by Silpisornkosol (1988), an abstract from which was presented in the proceedings of this meeting (Jenkins, 1989). Their research involved

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phenotypic ratios of F_1 , F_2 , and BC populations from crosses among 4 high glanding doubled haploid (DH) lines, various genetic stocks from Josh Lee, 'Stoneville 213' (ST213) and a glandless isoline of ST213. They proposed a genetic model that involved the interaction of the glanding loci, Gl2 and Gl3, and an inhibitor gene (I). In the homozygous dominant state (II), glanding on the calyx crown is inhibited, regardless of alleles present at the Gl loci. In the homozygous recessive state (ii), glands are expressed on the calyx crown if one or more dominant alleles are present at the Gl_2 or Gl_3 loci. Plants heterozygous at the inhibitor locus (Ii) have glands on the calyx crown if the genotype at the Gl loci is any combination of Gl_2 __Gl_3 __. With a few exceptions, their data fit this model. Exceptions are as follows: 1) F_2 and BC ratios of DH126 x ST213 did not fit expected 3:1 or 1:1 ratios, respectively, 2) F_2 ratio of DH126 x EX9160 glandless did not fit the expected 39:25 ratio, 3) different DH lines gave conflicting evidence regarding the inhibitor alleles present in EX9162 (with a putative Gl genotype of Gl_2 Gl_2 Gl_3r Gl_3r), 4) F_2 ratios from all DH lines x EX9161 (with a putative Gl genotype of Gl_2 Gl_2 gl_3 gl_3) did not fit the proposed model. From information reported, the origin of the high glanding alleles in this material was unclear.

The most commercially promising source of high glanding is material derived from Lukefahr's strain, XG-15 (Lukefahr and Houghtaling, 1969). This source of high glanding was utilized in the highly productive cultivars, Hartz H1215, H1220 and H1244 (Calhoun and Jones, 1994). The objective of this study was to determine the inheritance of the high glanding trait present in the Louisiana State Univ (LSU). and MAFES-Delta Res. and Ext. Ctr. (DREC) breeding programs, presumably derived from XG-15.

Materials and Methods

In 1993, crosses were made in the field at the DREC between La HG660 and 'Deltapine 5415' (Cross M31), and between LA881512 and 'Stoneville 907' (Cross M47). La HG660 is a high glanding strain from the LSU breeding program (Jones et al., 1988). LA881512 is a high glanding strain derived from a cross between La HG063 (Jones et al., 1988) and Miscot T8-27 (Bourland and Bridge, 1988). Deltapine 5415 and Stoneville 907 are well known Delta cultivars with normal glanding (i.e. few if any glands on calyx crown). F_1 seed of these crosses were planted in the cotton winter nursery in Tecoman, Mexico in 1993 and harvested as selfed F_2 bulks in 1994. F_2 seed was planted in the field at the DREC in the summer of 1994 on rows spaced 40 in. apart and thinned to approximately 18 in. between plants. At about first bloom, 2 squares per plant were collected and examined under a stereo-microscope at 10x magnification and glands on the calyx crown were counted. Mean number of glands on the calyx crown was calculated for each plant. At the end of the season, plants were individually harvested. F_3 progeny rows from each F_2 plant were grown in the field at DREC in 1995 on 40-in rows in plots 40 ft. long. From each row, 25 squares were collected and examined under a stereo-microscope. Squares were separated into two classes: normally glanded (i.e. <5 glands on calyx crown) and greater than normal glanding.

In 1994, additional crosses were made (Table 1). Parents included DES119-5 (a nectariless, sub-okra-leaf, smooth leaf isoline of 'DES119' with normal glanding, obtained from W.M. Meredith, USDA-ARS, Stoneville, MS), Hartz H1215 (high glanding commercial cultivar), Suregrow 404 (normal glanding commercial cultivar), Lag 80-4 (a glandless breeding line obtained from Gerald Meyers, LSU, Baton Rouge, LA), and two monomeric (i.e. homozygous recessive at one Gl locus and homozygous dominant at the other locus) lines, XG-15gl_2Gl_3 and XG-15Gl_2gl_3 , developed by Josh Lee and obtained from Daryl Bowman, North Carolina State Univ., Raleigh, NC. Lee confirmed that the dominant Gl alleles in the monomeric lines were derived from Lukefahr's XG-15 (Lee, pers. com., 1995). F_1 seed from these crosses were grown at the cotton winter nursery in 1994-95 and harvested as described above. Parents, remnant F_1 seed of 1993 and 1994 crosses, and F_2 populations of 1994 crosses were grown in the field at DREC as described above and thinned to approximately 18 in. between plants. One square per plant was collected and examined under a stereo-microscope. Squares were separated into three categories: 1) normally glanded, 2) greater than normal glanding on calyx crown, and 3) glandless.

Chi-square values were calculated for genetic ratios. When testing ratios with 1 degree of freedom, Yates correction was used in calculating chi-squares.

Results and Discussion

Evidence for control by a single locus

Frequency distribution of number of glands on the calyx crown of F_2 populations grown in 1994 (Crosses M31 and M47) showed a continuous distribution (Fig. 1), and the distribution was different for the two crosses. The only discrete phenotypic classes were normally glanded (<5 glands on calyx crown) and greater than normally glanded. Segregation ratios for F_2 populations of M31 and M47, based on these classes were 3 high glanding:1 normal glanding (Table 2). These data did not contradict the model proposed by Jenkins et al. (1989).

Data from $F_{2:3}$ progeny rows of Crosses M31 and M47 fit a ratio of 1 high glanding:2 segregating:1 normal glanding (Table 3), consistent with a single gene model, but also consistent with the recessive inhibitor gene model proposed by Jenkins et al. (1989).

Data collected in 1995 from F_2 populations of Crosses N56, N59, and N62 (see Table 1 for pedigrees) also fit a ratio of

3 high glanding:1 normal glanding (Table 2). Data from Crosses N56 and N59 fit the 3:1 ratio quite well, while Cross N62 had more high glanding plants than expected, though the data still did not justify rejecting the hypothesis of 3:1 ratio at the 5% probability level. It is possible that some normal glanding plants were mis-classified due to the effect of genetic background. As will be seen later, there was considerable overlap in phenotype among true-breeding and heterozygous genotypes.

Evidence of a special Gl3 allele

Examination of the monomeric lines, planted in 1994 for use as parents in 1994 crosses, revealed that XG-15gl_2Gl_3 was high glanding. This suggested that high glanding was conferred by an allele at the Gl_3 locus transferred from Socorro Island (a wild G. hirsutum), via XG-15 (Lukefahr and Houghtaling, 1969). Data from F_2 populations in 1994 supported the hypothesis of a Socorro Island Gl_3 allele conferring high glanding, which became our working hypothesis at this point. The proposed Socorro Island allele will be abbreviated in the following as Gl_3s.

We first confirmed with Dr. Josh Lee that dominant Gl alleles in the monomeric lines were, to the best of his knowledge, derived from the Socorro Island cotton. We next needed to confirm that the seed we received of monomeric lines were, in fact, momonmeric. Squares from the F_1 of Lag 80-4 x XG-15gl_2Gl_3 (Cross N67), with putative genotype, $gl_2gl_2Gl_3sgl_3$ were glandless; that is, no glands were visible on any part of the calyx, although glands may have been present on other plant parts. The F_2 population segregated 3 glandless (by the definition above):1 high glanding (Table 2). In this case, "high glanding" signified that if squares were glanded at all, they always had glands on the calyx crown; glands were often small and sparsely distributed. Expected F_2 phenotypes were: 1) glandless [2(gl_2gl_3) and $gl_2gl_2Gl_3sgl_3$] and 2) high glanding like XG-15gl_2Gl_3. The high glanding squares examined in the F_2 ranged from the XG-15gl_2Gl_3 phenotype to very small and sparsely distributed glands. The variation in high glanding phenotype could have been due to the effect of modifier genes or due to environmental factors. Several researchers have noted environmental and/or fruiting position effects on expression of other alleles at the Gl_2 and Gl_3 loci (e.g. White et al., 1982).

The F_1 of XG-15Gl_2gl_3 x glandless parents was also glandless according to the definition given previously. The F_2 population segregated 3 glandless:1 normally glanded (Table 2). In this case, "normal" signified that glands were restricted to the lower portion of the calyx, though they were often smaller and more sparsely distributed than normal.

The cross of XG-15gl_2Gl_3 with a normal glanding line (Table 1, Cross N69) produced high glanding F_1 plants, and an F_2 population that segregated 3 high glanding:1 normal glanding (Table 2). Thus, the genes in XG-15gl_3Gl_3 and in dimeric high glanding parents behaved similarly. The cross of XG-15gl_2Gl_3 with dimeric high glanding (Table 1, Cross N66) produced all high glanding plants in the F_1 and F_2 (Table 2). This observation supports the hypothesis that the high glanding monomeric line and the high glanding dimeric line share common alleles for high glanding, since this cross did not segregate any normal glanding plants.

Problems with the glandless x high glanding cross

One of the most compelling arguments for Jenkins' (1989) model is how well F_2 ratios from most high glanding x glandless crosses fit the expected 39:25 ratio. Our high glanding x glandless crosses (Table 1, Crosses N57 and N60) produced nearly glandless F_1 plants (i.e. only small, sparsely distributed glands on the lower portion of the calyx). Squares from the F_2 population ranged from glandless to high glanding, and literally everything in between. Expected genotypic and phenotypic ratios, based on our hypothesis of a Gl_3s allele for high glanding, are shown in Table 4. Observed and expected segregation ratios for Crosses N57 and N60 are shown in Table 5. Probability values from chi-square were in the rejection region for both crosses and for the combined data. Neither did our data fit the 39:25 ratio proposed by Jenkins (1989).

The failure of the data to fit the proposed genetic model could be due to either of two possibilities: 1) the model is incorrect, or 2) we were unable to correctly identify various genotypes due to over lapping phenotypes caused by incomplete penetrance or other factors. I believe this second possibility is particularly true when one or more glandless alleles are present at both Gl loci. We have harvested seed from individual F_2 plants from Crosses N57 and N60 and will observe $F_{2:3}$ progeny rows in 1996. Observation of $F_{2:3}$ progeny rows from high glanding x normal glanding crosses was useful understanding the range of phenotypes that can be expressed by a single genotype.

Considerations in selecting for high glanding

Regardless of the true genetic control of the high glanding trait, this study provided useful practical information for breeding high glanding cultivars. We demonstrated that in crosses of high glanding x normal glanding parents, it is possible to select true-breeding high glanding plants with fair success as early as the F_2 .

In Cross M31, F_3 progeny rows that were pure for high glanding came from F_2 plants with 79 to 22 glands on calyx crowns. Progeny rows that were pure for normal glanding came from F_2 plants with 5 or fewer (most with 2 or fewer) glands on the calyx crown, and segregating progeny rows came from F_2 plants with 2 to 55 (most between 10 and 30) glands on the calyx crown. In Cross M47, true-breeding high glanding F_2 plants had 98 to 30 glands on the calyx crown. All but one true breeding normal glanding F_2 plant had fewer than 5 glands on the calyx crown (the exception had 14), and segregating F_2 plants had from 2 to 57 (most

between 20 and 40) glands on the calyx crown. Visual identification of high glanding squares can be done in the field. Selection of plants with many glands on the calyx crown will usually result in offspring that are true-breeding for the high glanding trait, or at least offspring that will segregate high glanding.

If, in fact, our hypothesis of a single high glanding allele at the Gl_3 locus is true, it opens the possibility of developing insect resistant varieties using the high glanding trait without dramatically increasing (and possibly even decreasing) seed gossypol. Lee (1968) found that the Gl_2 locus contributed two times as much gossypol to seeds as the Gl_3 locus. He was working with the Gl_3 allele native to cultivated G. hirsutum, as opposed to our proposed Gl_3s allele. The effect of a possible Gl3s allele on seed gossypol content is as yet unknown. However, if our hypothesis is true, it would be possible to develop cultivars of the $gl_2gl_2Gl_3sGl_3s$ genotype that would be high glanding but lack the Gl_2 alleles that appear so potent in contributing to seed gossypol content.

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Table 1. Crosses made at DREC in 1994 to study inheritance of the high glanding trait.

Pedigree	Cross ID	Cross type[1]
La HG660 x DES119-5	N56	H x N
Hartz H1244 x DES119-5	N59	H x N
La HG660 x Suregrow 404	N62	H x N
Hartz H1244 x XG-15 gl2Gl3	N66	H x Gl3
DES119-5 x XG-15 gl2Gl3	N69	N x Gl3
Lag 80-4 x XG-15 gl2Gl3	N67	gl x Gl3
Lag 80-4 x XG-15 Gl2gl3	N68	gl x Gl2
Lag 80-4 x La HG660	N57	gl x H
Hartz H1244 x Lag 80-4	N60	H x gl

[1] H=high glanding, N=normal glanding, gl=glandless, Gl2=2(Gl_2gl_3), Gl3=2(gl_2Gl_3)

Table 2. Segregation ratios of F_2 populations from crosses involved in a high glanding inheritance study at Stoneville, MS in 1994 and 1995.

Cross	Phenotype[1] Observed		Expected (3:1)		P
High glanding (H) x normal glanding (N)					
	H	N	H	N	
M31	177	73	187.5	62.5	0.2 to 0.1
M47	189	52	180.8	60.3	0.5 to 0.2
N56	238	81	239.3	79.8	>0.95
N59	137	45	136.5	45.5	>0.95
N62	169	40	156.8	52.3	0.1 to 0.05
Glandless (gl) x monomeric XG-15gl2Gl3 (H)					
	gl	H	gl	N	
N67	576	179	566.3	188.8	0.5 to 0.2
High glanding (H) x monomeric XG-15gl2Gl3 (H)					
	H	N	H	N	
N66	97	0	97	0	--
Normal glanding (N) x monomeric XG-15gl2Gl3 (H)					
	H	N	H	N	
N69	174	50	168	56	0.9 to 0.5
Glandless (gl) x monomeric XG-15 Gl2gl3 (N)					
	gl	N	gl	N	
N68	90	30	90	30	0.95 to 0.90

[1] H=high glanding, N=normal glanding, gl=glandless

Table 3. Segregation ratio in $F_{2:3}$ progeny rows of high glanding (H) x normal glanding (N) crosses in Stoneville, MS, 1995.

	N	Segregating	H	P
Cross M31 (La HG660/Deltapine 5415)				
Observed	36	102	41	
Expected (1:2:1)	44.8	89.5	44.8	0.5 to 0.1
Cross M47 (LA881512/Stoneville 907)				
Observed	27	54	31	
Expected (1:2:1)	28	56	28	0.9 to 0.5

Table 4. Expected genotypic and phenotypic frequency from a cross of high glanding x glandless parents, assuming an allele at the Gl_3 locus (Gl_3s) transferred from Socorro Island cotton via the strain XG-15.

Proposed genotype	Frequency	Phenotype[1]	Phenotype based on[2]:
Genotypic frequencies			
Gl2 Gl2 Gl3s Gl3s	1	H	H parent
Gl2 gl2 Gl3s Gl3s	2	H	F1 (Gl3s x H)
gl2 gl2 Gl3s Gl3s	1	H	Gl3s monomeric parent
Gl2 Gl2 Gl3s gl3	2	unknown	F1 (Gl2 x H), no data
Gl2 gl2 Gl3s gl3	4	light basal	F1 (H x gl)
gl2 gl2 Gl3 gsl3	2	gl	F1 (Gl3 x gl)
Gl2 Gl2 gl3 gl3	1	N	Gl2 monomeric parent
Gl2 gl2 gl3 gl3	2	gl	F1 (Gl2 x gl)
gl2 gl2 gl3 gl3	1	gl	gl parent
Phenotypic frequency if unknown = H			
	6	H	
	4	light basal	
	1	N	
	5	gl	
Phenotypic frequency if unknown = N			
	4	H	
	4	light basal	
	3	N	
	5	gl	

[1] H=high glanding, N=normal glanding, gl=glandless, light basal=few small glands on base of calyx.
[2] Gl2=XG-15Gl_2gl_3, Gl3s=XG-15gl_2Gl_3

Table 5. Segregation ratio in F_2 populations of high glanding x glandless crosses at Stoneville, MS in 1995, and chi-squared tests assuming 2 possible phenotypic ratios.

	Phenotype[1] gl	light basal	N	H	P
	Assuming 5:4:1:6 ratio				
Cross N57					
Observed	74	74	27	46	
Expected (5:4:1:6)	69.06	55.25	13.8	82.88	>0.01
Expected (5:4:3:4)	69.06	55.25	41.44	55.25	>0.01
Cross N60					
Observed	51	57	12	33	
Expected (5:4:1:6)	47.81	38.25	9.57	57.37	>0.01
Expected (5:4:3:4)	47.81	38.25	28.69	38.25	>0.01
Combined					
Observed	125	131	39	79	
Expected (5:4:1:6)	116.88	93.50	23.40	140.25	>0.01
Expected (5:4:3:4)	116.88	93.50	70.13	93.5	>0.01

[1] H=high glanding, N=normal glanding, gl=glandless, light basal=few small glands on base of calyx.

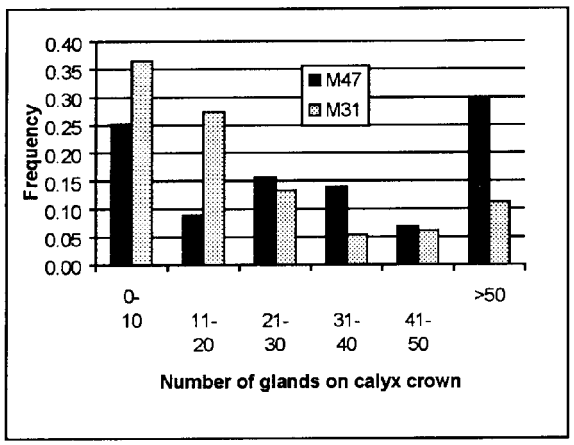

Figure 1. Frequency distribution of number of glands on calyx crown of F_2 populations of Cross M31 (La HG660/Deltapine 5415) and Cross M47 (LA881512/Stoneville 907) at Stoneville, MS in 1994.