Cotton *Gossypium* Spp. Plant Gossypol Contents of Selected GL_2 and GL_3 Alleles

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The gossypol content of cotton *Gossypium* spp. has been of interest to plant breeders because those that have high levels often carry resistance to the tobacco budworm *Heliothis virescens* (F.). However, its presence in seed has economic disadvantages to the seed and oil processing industry. Previously, two alleles which control plant gossypol, GL_2 and GL_3, were successfully transferred from several sources to a common background, XG-15. GL_3 has now been transferred from *G. raimondii* (Ulbr.), a Peruvian wild diploid species, and *G. davidsonii* (Kell), a Mexican wild diploid species, into *G. hirsutum* (Upland) cottons. The plant bud tissue resulting from these crosses is relatively high in gossypol, while seed is relatively low.

Keywords: *Cotton; Gossypium spp.; plant gossypol; alleles*

INTRODUCTION

The gossypol content of cotton *Gossypium* spp. has been of interest to plant breeders because those that have high levels often carry resistance to the tobacco budworm *Heliothis virescens* (F.). However, its presence in seed has economic disadvantages to the seed and oil processing industry. It is of interest to determine whether the gossypol content of various plant tissues on which the insect feeds and develops could be increased without increasing or by even decreasing seed gossypol.

The two main alleles that control the plant gossypol content are GL_2 and GL_3 (Bell and Stipanovic, 1977). Lee (1973, 1974) successfully transferred the GL_2 and GL_3 alleles from several sources to a common background XG-15. Lee et al. (1968) also reported that upland cotton plants monomeric for GL_2 produced more gossypol in seeds than those monomeric for GL_3; however, the disparity in seed gossypol production between cultivars of *G. barbadense* L. and the highest of the *G. hirsutum* L. stocks seems attributable to the greater expressivity of the GL_3 allele in the upland cottons.

Lukefahr and Martin (1966) and Wilson and Shaver (1973) reported that raising the terpenoid level in flower buds (squares) provided resistance to *Heliothis* spp. in upland cottons. It would appear that transfer of the GL_2 and GL_3 alleles from various available sources to upland cultivars could increase the gossypol content and provide insect resistance.

This study is an investigation of the content of gossypol and other allelochemicals in leaves, squares, bracts, and seeds of 14 experimental lines, some for the first time, with one cultivar included as a baseline comparison.

MATERIALS AND METHODS

Cotton Sources. Cotton lines with various sources of the Gl_2 and Gl_3 alleles were tested. The Gl_2 alleles were transferred from the following sources: (1) a high gossypol strain of *G. hirsutum*; (2) Coker 310, an upland cultivar adapted for production in the South Atlantic states; and (3) H-1104, a "dooryard" *G. hirsutum*. The Gl_3 allele was transferred from the following sources: (1) *G. mustelinum* Miers ex Watt, a Brazilian wild tetraploid; (2) *G. tomentosum* Nutt. ex Seem, a Hawaiian wild tetraploid; (3) Coker 310; 4) 3-T, an experimental strain created by M. J. Lukefahr (Lee, 1977) that has exceptionally high seed gossypol but relatively low flower bud gossypol; (5) *G. raimondii* Ulbr., a Peruvian wild diploid species; (6) *G. davidsonii* Kell., a Mexican wild diploid species; and (7) H-1014, a "dooryard" *G. hirsutum* from India. XG-15 is a high-gossypol experimental strain (Lukefahr and Houghtaling, 1969) of *G. hirsutum* that is the sibling of 3-T. This line harbors the Gl^r (rugate allele), a gene that conditions high boll glandulosity (Lee, 1978). Five to seven backcrosses were used to transfer Gl_3 alleles to the XG-15 background. In addition to the above strains, monomeric (Gl_2Gl_2 gl_3gl_3 and gl_2gl_2 Gl_3rGl_3r), nullomeric (glandless, gl_2gl_2 gl_3gl_3), and dimeric XG-15 (Gl_2Gl_2 Gl_3rGl_3r) strains and Stoneville 213 (Gl_2Gl_2 Gl_3Gl_3), a commercial cultivar, were included in the test (Table 1).

1982−1994 Field Tests. The field tests were conducted in 1982, 1983, 1989, 1991, and 1994 on the Plant Science Farm at Mississippi State University. The cotton was planted each year about May 1 in single-row [0.97 × 12.8 m (W × L)] plots. Insects were controlled, as needed, with malathion and a synthetic pyrethroid.

Analyses of Allelochemicals. Plant tissues (ca. 25 terminal leaves, squares, and bracts from each of two replications) were collected, freeze-dried, and ground. Allelochemical analyses were carried out following the procedures of Hedin et al. (1983a, 1991) for gossypol (phloroglucinol), flavonoids (diphenylboric acid−ethanalamine complex), and anthocyanins (absorbency at 540 nm of an aqueous alcoholic extract). Phloroglucinol was used for the analysis of gossypol as a substitute for aniline, which may be a health hazard. The results of the gossypol analyses were similar (Hedin et al., 1983b). The analyses were conducted in duplicate for each replication. Analyses were also performed using the anthrone procedure for total free sugars (Hedin et al., 1991). About 50 seeds were dehulled and ground prior to analysis for gossypol.

Statistical Procedures. Data obtained from the various analyses and measurements were subjected to an analysis of variance, and lsd values were calculated according to SAS Institute (1985) methods.

RESULTS AND DISCUSSION

In 1982 and 1983, preliminary field tests and analytical work were carried out on several lines in which the

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Table 1. Entry Number, Genotype, Source of Gl_2 or Gl_3 Alleles of Cotton Strains, and Abbreviations Used in Tables

entry	genotype	source of Gl_2 or Gl_3 allele	abbrev
1	gl_2gl_2 Gl_3Gl_3	*G. davidsonii*	Gl_3 dav.
2	gl_2gl_2 Gl_3Gl_3	*G. tomentosum*	Gl_3 tom.
3	gl_2gl_2 Gl_3Gl_3	*G. mustelinum*	Gl_3 mus.
4	gl_2gl_2 Gl_3Gl_3	*G. hirsutum* cv. Coker 310	Gl_3 C310
5	gl_2gl_2 Gl_3Gl_3	*G. hirsutum* dooryard H1104	Gl_3 H1104
6	gl_2gl_2 Gl_3Gl_3	*G. hirsutum* strain 3-T	Gl_3 3-T
7	gl_2gl_2 Gl_3Gl_3	*G. raimondii*	Gl_3 rai.
8	Gl_2Gl_2 gl_3gl_3	*G. hirsutum* high-gossypol strain	Gl_2 h goss.
9	Gl_2Gl_2 gl_3gl_3	*G. hirsutum* cv. Coker 310	Gl_2 C310
10	Gl_2Gl_2 gl_3gl_3	*G. hirsutum* dooryard H1014	Gl_2 H1014
11	Gl_2Gl_2 gl_3gl_3		2(Gl_2gl_3)
12	gl_2gl_2 Gl_3rGl_3r		2(gl_2Gl_3r)
13	Gl_2Gl_2 Gl_3rGl_3r	strain XG-15	XG-15
14	gl_2gl_2 gl_3gl_3	Stoneville 213 glandless	ST-213 gl
15	Gl_2Gl_2 Gl_3Gl_3	Stoneville 213	ST-213

Table 2. Gossypol in Cotton Leaves, Squares, and Seed in 1982 and 1983 (Percent)[a]

		small leaves		flower buds		seed
no.	entry	1982	1983	1982	1983	1982
1	Gl_3 dav.	0.16	0.17	0.22	0.40	0.68
2	Gl_3 tom.	0.16	0.14	0.09	0.14	0.71
3	Gl_3 mus.	0.15	0.17	0.08	0.15	0.78
5	Gl_3 H1104	0.14	0.16	0.12	0.20	0.34
6	Gl_3 3-T	0.20	0.16	0.10	0.23	0.50
7	Gl_3 rai.	0.21	0.21	0.16	0.40	0.80
13	XG-15		0.35		0.67	
14	ST-213 gl	0.13	0.17	0.16	0.09	0.10
15	ST-213	0.25	0.23	0.23	0.52	

[a] A preliminary test with insufficient replication for statistical evaluation, percent of dry weight.

Gl_2 and Gl_3 alleles were transferred from various sources to a common background (Table 1). There was insufficient replication for statistical evaluation. The Gl_3 allele from *G. davidsonii* and *G. raimondii* gave leaf and square gossypol levels comparable to those of the commercial cultivar Stoneville 213 (Table 2). The seed gossypol of the Gl_3 entries ranged from 0.34 to 0.80%, whereas Lee (1977) had reported levels of 1−3% from several Gl_2 and Gl_3 lines. This difference should be attributed to the sources of plant material because results with the two gossypol reagents were similar (Hedin et al., 1983b). The Gl_3 allele from *G. davidsonii* gave the best combination of square (0.22−0.40%) and seed (0.68%) gossypol. The other sources of the Gl_3 allele did not seem to hold promise for increasing square gossypol while limiting the quantity in seed. Square gossypol was only slightly increased by the transfer of Gl_2 sources. The leaf gossypol levels were only slightly higher than that of glandless levels in both 1982 and 1983. The square gossypol levels of the GL_2 sources were about equal to the glandless levels in 1982 but were considerably higher in 1983. None of the GL_2 sources appeared to be useful for increasing bud gossypol. The Gl_3 allele from *G. davidsonii* and *G. raimondii* produced gossypol levels in flower buds that approached that of the cultivar ST-213.

Tests on the effects of the Gl_2 and Gl_3 alleles on square and leaf gossypol were continued in 1989 and 1991. The results are summarized in Table 3. Analyses of the content of gossypol in seeds were performed in 1991 but not in 1989. Square gossypol was high both years in *G. davidsonii* and *G. raimondii*, sources of the GL_3 allele. Both the monomeric gl_2gl_2 Gl_3rGl_3r and dimeric XG-15 resulted in increased square gossypol relative to ST-213. This is in agreement with previously published

Table 3. Total Gossypol in Cotton Plant Leaves, Squares, and Seeds, 1989 and 1991 (Percent)[a]

			1991		
no.	entry	1989 square gossypol	square gossypol	leaf gossypol	seed gossypol
1	Gl_3 dav.	0.56	0.35	0.28	0.69
2	Gl_3 tom.	0.17	0.26	0.21	0.61
3	Gl_3 mus.		0.25	0.20	0.65
4	Gl_3 C310	0.24	0.24	0.16	0.50
5	Gl_3 H1104	0.17	0.21	0.12	0.38
6	Gl_3 3-T	0.27	0.32	0.14	0.45
7	Gl_3 rai.	0.73	0.66	0.24	0.72
8	Gl_2 h goss.	0.23	0.27	0.18	0.61
9	Gl_2 C310	0.20	0.31	0.20	0.59
10	Gl_2 H1014	0.40	0.34	0.16	0.44
11	2(Gl_2gl_3)	0.32	0.26	0.26	0.61
12	2(gl_2Gl_3r)	0.60	0.46	0.20	0.78
13	XG-15		0.79	0.53	1.02
14	ST-213 gl	0.04	0.07	0.13	0.04
15	ST-213	0.46	0.42	0.28	0.81
	lsd 0.05	0.04	0.05	0.05	0.06

[a] Phloroglucinol procedure.

data (Lee, 1977, 1978). Transfer of the Gl_2 allele into the *G. hirsutum* lines did not result in appreciable increases in square gossypol, however. Leaf gossypol also was not appreciably increased upon transfer of the Gl_2 allele to the *G. hirsutum* lines.

In cotton plant tissues, gossypol is often the most prevalent of several terpenoid aldehydes (Stipanovic et al., 1984, 1988; Hedin et al., 1991). However, in seeds, gossypol is virtually the only terpenoid aldehyde present (Stipanovic et al., 1988; Khoshkhoo et al., 1994). For this work, it was considered of interest to compare the results of a reasonably specific colorimetric procedure, phloroglucinol (Hedin et al., 1991), to the HPLC procedure of Stipanovic et al. (1988) to determine whether recoveries from squares were similar and whether there was some unusual distribution of terpenoid aldehydes in any of the lines.

Analysis of gossypol in squares of the 1994 lines by the phloroglucinol procedure gave somewhat lower, often variable, results that averaged 65% of the HPLC procedure (Table 4). Although the HPLC procedure is obviously more precise, the phloroglucinol procedure appears to be acceptable for preliminary screening because a plot of the phloroglucinol versus HPLC data was generally linear, particularly for the lower and higher analyses. These are the analyses of greatest interest. The ratios of the terpenoid aldehydes were fairly constant except for the lines having total terpenoid aldehyde (TTA) content quite different from the phloroglucinol value, which had higher heliocide or hemigossypolone (HGO) values compared with the GL_3 dav. line.

The TTA and phloroglucinol values for GL_3 dav. and its HGO and heliocide values were fairly low. With the HPLC procedure, square gossypol accounted for 50−82% of the terpenoid aldehydes in the analyzed lines. Of the other components, hemigossypolone accounted for 3−8%, heliocide H_1 6−17%, H_2 4−13%, H_3 2−5%, and H_4 3−8%. From these data, it appears that the colorimetric analysis is adequate to evaluate the effects of the various alleles on the resulting gossypol content in squares.

Tests to determine the effects of Gl_2 and Gl_3 alleles on plant tissue and seed gossypol contents were also carried out in 1994. In addition, analyses of two other insect allelochemicals, anthocyanins and flavonoids,

Table 4. Total Gossypol in 1994 Cotton Squares As Analyzed by the Phloroglucinol Procedure and Total Terpenoid Aldehydes As Determined by Summation of the HPLC Maxima (Percent)[a]

		square terpenoid aldehydes, HPLC analysis[b]							
no.	entry	gossypol[c]	HGO	G	H_1	H_2	H_3	H_4	TTA
1	Gl_3 dav.	0.246	0.007	0.218	0.016	0.011	0.005	0.008	0.265
2	Gl_3 tom.	0.265	0.027	0.225	0.042	0.040	0.013	0.019	0.366
3	Gl_3 mus.	0.252	0.030	0.257	0.059	0.054	0.018	0.026	0.444
4	Gl_3 C310	0.102	0.015	0.094	0.019	0.018	0.006	0.009	0.161
5	Gl_3 H1104	0.174	0.021	0.149	0.039	0.036	0.013	0.017	0.275
6	Gl_3 3-T	0.236	0.038	0.277	0.042	0.047	0.014	0.019	0.437
7	Gl_3 rai.	0.350	0.024	0.401	0.081	0.051	0.018	0.039	0.614
8	Gl_2 h goss.	0.270	0.031	0.328	0.073	0.056	0.019	0.033	0.540
9	Gl_2 C310	0.366	0.028	0.365	0.039	0.042	0.014	0.017	0.505
10	Gl_2 H1014	0.376	0.028	0.358	0.064	0.050	0.017	0.028	0.545
11	$2(GL_2gl_3)$	0.255	0.013	0.264	0.045	0.035	0.012	0.020	0.389
12	$2(gl_2Gl^3r)$	0.361	0.020	0.508	0.074	0.047	0.017	0.034	0.700
13	XG-15	0.529	0.097	0.726	0.229	0.158	0.049	0.112	10.371
14	ST-213 gl	0.030	0.001	0.013	0.004	0.003	0.002	0.003	0.026
15	ST-213	0.310							

[a] Analysis expressed to three decimal places to accommodate HPLC data. [b] HGO, hemigossypolone; G, gossypol; H_1, H_2, H_3, and H_4, heliocides, TTA, total terpenoid aldehydes. [c] Phloroglucinol procedure.

Table 5. Total Gossypol in Leaves, Squares, Bracts, and Seeds of 1994 Cottons (Percent)

no.	entry	square gossypol	leaf gossypol	bract gossypol	seed gossypol
1	Gl_3 dav.	0.20	0.13	0.05	
2	Gl_3 tom.	0.22	0.13	0.05	
3	Gl_3 mus.	0.20	0.14	0.06	0.71
4	Gl_3 C310	0.18	0.12	0.04	
5	Gl_3 H1104	0.19	0.12	0.07	0.55
6	Gl_3 3-T	0.22	0.14	0.05	0.52
7	Gl_3 rai.	0.32	0.21	0.09	0.46
8	Gl_2 h goss.	0.23	0.13	0.08	0.77
9	Gl_2 C310	0.32	0.14	0.06	0.60
10	Gl_2 H1014	0.33	0.17	0.07	0.59
11	$2(Gl_2gl_3)$	0.27	0.17	0.07	0.59
12	$2(gl_2Gl_3r)$	0.37	0.14	0.07	0.84
13	XG-15	0.56	0.27	0.16	0.95
14	ST-213 gl	0.05	0.06	0.06	0.14
15	ST-213	0.33	0.16	0.08	0.70
	lsd 0.05	0.046	0.023	0.017	0.03

[a] Average of two sampling dates, Aug 4, 1994, and Aug 12, 1994, phloroglucinol procedure.

were carried out on plant tissue. The total sugar contents were also determined.

The results of the analyses by the phloroglucinol procedure for total square gossypol in 1994 cottons (Table 5) were generally comparable to those performed in the previous years (Tables 2 and 3). The transfer of the Gl_2 and Gl_3 alleles to *G. hirsutum* lines did not result in higher gossypol. Total leaf, bract, and seed gossypol contents also were not markedly increased.

Anthocyanins were analyzed because they had been shown to exist in part as a halo around gossypol glands (Hedin et al., 1983a) and had also been shown to be toxic to the tobacco budworm *H. virescens* (Fab.) in their own right. However, squares of all lines tested were low in anthocyanins (average 0.07%) except for XG-15 $2(Gl_2$-$Gl_3)$ (0.13%). Similarly, only this line possessed higher anthocyanin levels in leaves (0.55%; average 0.27%) and bracts (0.24%; average 0.14%). All lines were also analyzed for their flavonoid and sugar contents, but no helpful trends were identified. The average contents of flavonoids were as follows: squares, 0.37%; leaves, 1.04%; and bracts, 0.46%. The average contents of sugars were as follows: squares, 9.91%; leaves, 12.59%; and bracts, 11.89%. These data suggest that the alleles responsible for the increased gossypol content may not cause increases of other allelochemicals or metabolites.

Selective combinations of gland-determining alleles can reduce (unfavorably) high amounts of toxic gossypol in cotton seed without reducing amounts in flower buds (Wilson and Shaver, 1973). These authors suggested that the genotype $2(gl_2Gl_3)$ be used, in which the source of Gl_3 is *G. raimondii*. They had shown that this genotype reduced *H. virescens* larval growth without elevating seed gossypol levels. Our data would also suggest the efficacy of using Gl_3 from both *G. raimondii* and *G. davidsonii*. These alleles gave flower bud gossypol levels that were equivalent to that of ST-213 but did not elevate seed gossypol levels.

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