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Genetic Analysis of Egyptian Glandless Cotton[1]

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ABSTRACT

The Egyptian cotton (*Gossypium barbadense* L.) strain, 'Bahtim 110', is glandless due to the expression of a dominant allele at a single locus while the glandless condition found in Upland cotton (*G. hirsutum* L.) is due to the expression of recessive alleles at two loci. The gene conditioning the glandless character in the Egyptian strain was found to be an allele at the Gl_2 locus and was designated Gl_2^e. Potential use of this gene in glandless cottonseed breeding programs is discussed.

***Additional index words:* *Gossypium barbadense* L., *G. hirsutum* L., Linkage, Monosomes, Gossypol, Cottonseed breeding.**

McMichael (1959) reported the identification of a mutant in cotton in which the aerial plant parts were devoid of the lysigenous pigment glands typical of *Gossypium* spp. The glandless phenotype was determined by the combined action of the recessive genes gl_2 and gl_3 in the homozygous state (McMichael, 1960). Egyptian workers reported the identification of a single dominant mutation in *G. barbadense* L., following the irradiation of 'Giza 45' seed with ^{32}P, that imparted glandlessness. A strain of cotton homozygous for this new allele was released as 'Bahtim 110' (Afifi et al., 1965; 1966). Seed designated as Bahtim 110 were distributed to U.S. cotton workers. We began genetic analyses independently, but we combined our efforts when we became aware of each other's activities. This paper reports the combined results of these genetic analyses.

MATERIALS AND METHODS

J.A. Lee performed genetic analyses in the *G. barbadense* background. The tester stocks that were used were derived from 'Sea Island' with the genotypes $2(Gl_2Gl_3)$, $2(Gl_2gl_3)$, and $2(gl_2Gl_3)$, and 'Pima S-4' derived $2(gl_2Gl_3)$ and $2(gl_2gl_3)$. R.J. Kohel transferred the glandless mutant to *G. hirsutum* by backcrossing to TM-1 (Kohel et al., 1970). Three and four backcrosses were completed with the glandless mutant "isoline" that was used in this study. All tester lines were *G. hirsutum*, except for one involving the doubled haploid experimental strain of *G. barbadense*, 3-79. The *G. hirsutum* standard, TM-1, has the genotype $2(Gl_2Gl_3)$. The tester, $2(gl_2gl_3)$, and the monosome for chromosome 12, were TM-1 isolines. The monomeric $2(Gl_2gl_3)$ was obtained from J.A. Lee. Linkage analyses utilized T582 and T586, recessive and dominant multiple marker tester lines, respectively (Kohel, 1978).

RESULTS AND DISCUSSION

Inheritance

Crosses of the glandless Bahtim 110[$2(Gl^e)$] to normally glanded Sea Island (SI) and 3-79 produced F_1 plants with reduced glandedness on the cotyledons. The F_2 segregation ratios confirmed that the segregation was that of a single partially dominant gene (Table 1).

Crosses of the glandless Bahtim 110 to normally glanded *G. hirsutum*, TM-1, produced a variable response. Cotyledonary classification for presence or absence of glands based on that established by Lee (1962) for the segregation of the gl_2 and gl_3 genes was used. Initially it was not clear whether the variable segregation pattern was due to the instability of the interspecific hybrid population, genetic modifiers, or a less distinct phenotypic expression associated with this mutant than that reported for gl_2 and gl_3 by Lee (1962). With additional backcrosses (BC) and progeny testing (Table 1) it was established that the segregation was that of a single partially dominant gene and that the heterozygote had a range of expression of glandedness. Cotyledons of heterozygous plants had either marginal or reduced glandedness while hypocotyls had either reduced or no glands. Subsequent heterozygous progeny from both the marginal and reduced glandedness classes gave both types of phenotypic expression in the cotyledons. Therefore, the phenotype of the heterozygote is described as sparse cotyledonary glandedness to include this range of variability, and to distinguish it from the phenotypic classes associated with segregation of the recessive gl_2 and gl_3 genes.

Allelism Tests

Once the mode of inheritance of the Bahtim 110 glandless mutant reported by Afifi et al. (1965,1966)

Table 1. Segregation of glandless Bahtim 110, $2(Gl^e)$, following crosses with normally glanded *G. barbadense* and *G. hirsutum* cottons.

Phenotype	No. plants			Segregation Pooled	Proposed ratio	Chi-square and probability
G. barbadense						
	$[2(Gl^e)\times SI]F_2$,			$[3\text{-}79\times 2(Gl^e)]F_2$		
	a	b				
Glandless	50	42		92	1	$X^2 = 0.59$
Reduced	112	88		200	2	P = 0.80–0.70
Glanded	56	46		102	1	
				394		
G. hirsutum						
	$[TM\text{-}1\times 2(Gl^e)]F_2$					
	a	b		Pooled		
Glandless	17	47		64	1	$X^2 = 6.27$
Sparse	9	90		99	2	P = 0.05–0.02
Glanded	5	34		39	1	
				202		
	$TM\text{-}1[TM\text{-}1\times 2(Gl^e)]S_1BC$					
	a	b	c	Pooled		
Glandless	23	287	74	384	1	$X^2 = 1.82$
Sparse	53	552	110	715	2	P = 0.50–0.30
Glanded	24	306	53	383	1	
				1482		
	$TM\text{-}1[TM\text{-}1\times 2(Gl^e)]BC$					
	a	b	c	Pooled		
Sparse	12	15	50	77	1	$X^2 = 0.06$
Glanded	14	10	56	80	1	P = 0.90–0.80
				157		

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Table 2. Allelic tests of glandless Bahtim 110, 2(Gl^e), with gl_2 and gl_3 *G. barbadense* and *G. hirsutum* testers.

Phenotype	Segregation No. plants				Proposed ratio	Chi-square and probability
G. barbadense						
	[2(Gl^e) × 2(gl_2Gl_3)]F_2					
	SI	S-4		Pooled		
Glandless	133	82		215	3	X^2 = 0.41
Glanded	52	26		78	1	P = 0.70–0.50
				293		
	[2(Gl^e) × 2(Gl_2gl_3)]F_2					
Glandless	143				10	X^2 = 3.32
Sparse	37				2	P = 0.20–0.10
Glanded	71				4	
	251					
	[2(Gl^e) × 2(gl_2gl_3),S-4)]F_2					
Glandless	195				13	X^2 = 1.37
Sparse	33				2	P = 0.50–0.30
Glanded	11				1	
	239					
G. hirsutum						
	[2(Gl_2gl_3) × 2(Gl^e),TM-1]F_2					
Glandless	73				10	X^2 = 2.83
Sparse	9				2	P = 0.30–0.20
Glanded	22				4	
	104					
	[2(gl_2gl_3),TM-1 × 2(Gl^e)]F_2					
	a	b	c	Pooled		
Glandless	42	64	149	255	13	X^2 = 8.90
Sparse	7	11	37	55	2	P = 0.02–0.01
Glanded	6	13	11	30	1	
				340		
	(Monosome 12 × 2(Gl^e),TM-1]					
	F_1	F_2				
Glandless (monosome)	6	all glandless (7 plants)				
Glanded (disome)	17	segregation (1:2:1)				X^2 = 0.12 P = 0.95–0.90

was found to express in both *G. barbadense* and *G. hirsutum,* tests were made to determine the relation, if any, with the gl_2 and gl_3 loci. Bahtim 110 was crossed to the Sea Island monomerics, 2(Gl_2gl_3) and 2(gl_2Gl_3), and Pima S-4 monomeric, 2(gl_2Gl_3). In both monomeric cross combinations the F_1 seedlings had glandless cotyledons. Segregation of the F_2 from the crosses of the Gl_3 monomeric, 2(gl_2Gl_3), combination fit a 3 glandless to 1 glanded ratio (Table 2). This segregation suggested that the Bahtim 110 glandless mutant gene was an allele at the Gl_2 locus.

With the information from the Gl_3 monomeric F_2 and knowing the phenotypes of both monomerics × Bahtim 110 F_1's, our postulated ratio in the Bahtim 110 × Gl_2 monomeric, 2(Gl_2gl_3), F_2 was 10 glandless : 2 sparse : 4 glanded cotyledonary classes. The data fit this expected ratio (Table 2).

With the assumption that glandless Bahtim 110 also carries a Gl_3 allele, one can predict that crosses with the recessive glandless, 2(gl_2gl_3), would produce a seedling with cotyledons that were glandless in the F_1 and in the F_2 would have the phenotypic ratio of 13 glandless: 2 sparse : 1 glanded. Segregation of Bahtim 110 × Pima S-4 glandless, 2(gl_2gl_3), F_2 fit this expected ratio (Table 2).

The glandless Bahtim 110 allele, Gl^e, transferred to *G. hirsutum* (TM-1), was utilized in a similar series of tests with *G. hirsutum* testers. Crosses of 2(Gl^3) with the monomeric, 2(Gl_2gl_3), produced F_1 seedlings with glandless cotyledons, and the F_2 segregated with the ratios of the phenotypic classes 10 glandless : 2 sparse : 4 glanded (Table 2).

Table 3. Detailed analysis of Gl_2^e and N_1 linkage.

Phenotype	Segregation No. plants				Chi-square analysis		
[2(Gl_2N_1) × 2($Gl_2^en_1$)]2(Gl_2n_1)					Source	Chi-square	P
	a	b	c	Pooled			
$Gl_2^en_1$	49	24	14	87	Cl_2^e vs. Gl_2	= 1.00	0.50–0.30
$Gl_2^eN_1$	13	10	10	33	N_1 vs. n_1	= 2.78	0.10–0.05
Gl_2n_1	23	10	5	38	Linkage	= 30.61	<0.01
Gl_2N_1	32	13	22	67	Recombination percent =		
				225			31.56 ± 3.10

The Bahtim 110 allele, Gl^e, was crossed to the *G. hirsutum* glandless isoline, 2(gl_2gl_3), from its native background and after each of two backcrosses to TM-1. The segregation in the resulting three *G. hirsutum* F_2 seedling populations did not fit the 13 glandless : 2 sparse : 1 glanded phenotypic ratio as well as in the *G. barbadense* F_2 population (Table 2). However, all the other data show that Gl^e is an allele of Gl_2 and these data do not refute that finding.

The Bahtim 110 mutant is a partially dominant mutant allele at the Gl_2 locus. This single dominant allele mimics the same control of gland expression as that obtained by the combined effects of the duplicate recessive genes gl_2 and gl_3 discovered by McMichael (1959). We propose the gene symbol Gl_2^e to designate this new dominant allele at the Gl_2 locus.

Linkage and Monosome Analyses

Two additional tests were performed with the Gl_2^e TM-1 isoline. It was crossed with the monosome for chromosome 12 for verification of chromosome location, and with the multiple marker lines, T582 and T586, for linkage analysis. When the Gl_2^e isoline was crossed onto the monosome, the resulting F_1 seedlings segregated into glandless and sparse cotyledonary glandedness classes. When transplanted to the field the glandless plants expressed the monosome 12 phenotype and the sparse glanded plants expressed the normal disomic phenotype (Table 2).

When the crosses for the linkage analysis were begun, allelism of Gl_2^e to the Gl_2 locus was not known. The only genetic marker in the multiple marker lines that is resident on the same chromosome as Gl_2, chromosome 12, is N_1 (Naked seed). Therefore the linkage relation between Gl_2^e and N_1 is the test combination of primary interest. The linkage analysis results and detailed analysis of the Gl_2^e-N_1 linkage (Table 3) revealed a significant $Gl_2^eN_1$ linkage with 32% recombination. Recombination percentage between gl_2-N_1 was 37.61 ± 4.66 (Kohel, unpublished).

Practical Implications

The Gl_2^e allele offers a new alternative genetic system to glandless cottonseed breeding programs. Use of the Gl_2^e allele provides greater ease and simplicity in genetic manipulation because it is a single dominant gene as opposed to the previously used genetic system that involved the duplicate recessives gl_2 and gl_3. The single partially dominant gene would result in easier recognition in segregating populations and

a resulting decrease in population size and number of generations required for transference to different backgrounds.

The Gl_2^e allele may also be combined with gl_3 to solve another problem of producers of glandless cottonseed. The very low tolerance requirements for seed gossypol require stringent quality control. One source of contamination is through outcrossing with glanded cottons that produce glanded F_1 seeds. A glandless cotton that combined the Gl_2^e and gl_3 genes, $2(Gl_2^e gl_3)$, when crossed with a normally glanded cotton, $2(Gl_2Gl_3)$, would produce glandless F_1 seeds; the seedling genotype would be $Gl_2^eGl_2Gl_3gl_3$. This system could be useful in preventing contamination due to chance outcrossing with glanded plants in production fields.

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