Linkage Tests in Upland Cotton. III [1]

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ABSTRACT

A summary of tests of 255 linkage combinations are reported. Two new linkages were established as follows: Rd and v_8 (14.22% recombination; Linkage Group XIV) and Li and v_3 (10.12% recombination; Linkage Group XV). Eleven other suspected linkages are discussed, but they were either eliminated through monosomic analysis or were not adequately validated. In the latter case, current information is presented because these mutants are so difficult to manipulate that development of test populations will require several years of work.

Additional index words: Linkage groups, Chromosome identification.

RESEARCHERS in the cotton genetics program at College Station, Tex. use both genetic and cytogenetic methods to identify the 26 chromosome pairs of cotton (*Gossypium hirsutum* L.). Genetic identification of the chromosomes is pursued with the use of linkage and aneuploid analyses. This paper reports the results of linkage analyses performed since the last summary in 1972 (2).

MATERIALS AND METHODS

Names of the mutants, their gene symbols, and information regarding their chromosome location and/or linkage group are summarized in Table 1. In cotton, numbers 1 to 13 are assigned to the A subgenome chromosomes and numbers 24 to 26 are assigned to the D subgenome chromosomes. Linkage groups are identified by Roman numerals in chronological order of their identification (3).

The bulk of the mutants occurred as monogenic markers in individual lines of cotton. Most were tested with our multiple marker stocks T582 and T586, so, with the exception of some of the more recent accessions, linkage tests involved dihybrid combinations. One male-sterile mutant was digenic (ms_5ms_6). The three multiple marker lines used included one containing the three gland determining loci gl_1, gl_2, and gl_3 and the two multiple marker test lines T582 (cu, fg, cl_1, gl_1, and v_1) and T586 (R_2, Lc_1, L^o_2, R_1, H_2, Y_1, N_1, Lg, and P_1).

Linkage deviations were determined by chi-square analyses. Recombination values were computed by the product method (9) for dihybrid F_2 generation test combinations and the maximum likelihood method (1) for all other test combinations and pooled estimates.

RESULTS AND DISCUSSION

Results of the linkage tests are summarized in Table 2 (recessive × recessive mutant loci), Table 3 (recessive × dominant mutant loci), and Table 4 (dominant × dominant mutant loci). Data shown are recombination percentages, population sizes, generation tested, and significant linkage deviations. Two close linkages were found and verified by retesting.

The first association observed was that between Rd and v_8 (14.22% recombination; Table 5). Rd has been associated with the D subgenome (Table 1). No other linkage or chromosome associations have been reported for these mutant loci; therefore, they represent a new linkage group in cotton, Linkage Group XIV.

The second close linkage observed was that between Li and v_3 (10.12% recombination; Table 5). Tests involving these loci did not indicate any other associations. Therefore, Li and v_3 are designated Linkage Group XV.

Table 1. Name, gene symbol, chromosome or subgenome, linkage group, and reference of cotton mutants involved in linkage test.

Name	Gene symbol(s)	Chromosome or subgenome	Linkage group	Reference
Cluster fruiting-1	cl_1	16	III	2
Cup leaf	cu	A		2
Depauperate	de			4
Frego bract	fg		VI	2
Glandless stem and boll	gl_1	D		2
Glandless plant	gl_2, gl_3	A,D	V, IX	2
Inverted stigma-style	st_3			3
Mosaic leaf	ml	4	VIII	2
Male-sterile-2	ms_2			2
Male-sterile-5,6	ms_5, ms_6			3
Miniature stigma-style	st_2			3
Naked seed	n_2			3
Open bud	ob			3
Pale green	pg			3
Round leaf-1	rl_1		X	2
Virescent-1	v_1	A		2
Virescent-2	v_2			3
Virescent-3	v_3		XV	3, text
Virescent-4	v_4			3
Virescent-8	v_8	D	XIV	5, text
Withering bract-1	bw_1	A	V	2
Brown lint-1	Lc_1	7	I	2
Crumpled	Crp			3
Green lint	Lg	15	II	2
Laciniate leaf	Lf	1	VII	2
Ligon lintless	Li	D	XV	2, text
Male-sterile-4	Ms_4			2
Male-sterile-7	Ms_7			3
Naked seed	N_1	A	XIII	6
Okra leaf	L^o	15	II	2
Petal spot	R_2	7	I	2
Pilose	H_2	6	IV	2
Ragged leaf	Rg		X	2
Red dwarf	Rd	D	XIV	2, text
Red plant	R_1	16	III	2
Round leaf-2	Rl_2			8
Rugate	Ru			3
Smooth stem	Sm_1, S			3
Yellow petals	Y_1	A	XII	7
Yellow pollen	P_1		XI	10

Table 2. Summary of cotton linkage test results between lines carrying recessive mutants.†

	v_8	v_4	v_3	v_2	v_6	rl_1	pg	ob	n_2	ms_5ms_6	ms_2	ml	st_3
cl_1									44 138	50 98			55 92
cu									63 138	50 98			49 92
de	43 90		46 86	60 91							63 76		
fg									58 138	46 98			48 92
gl_1		41 136	42 98				39 55		51 75	49 98		45 144	
gl_2	58 116	46 217	60 66	40 65			57 60					53 208	
gl_3	64 116	44 217	56 66	48 65			67 60					57 208	
st_3	37 93	53 91	53 176	39 93	53 92	40 97	45 86	51 83			50 87		
ml		52 79		58 208		63 60	58 71	54 87					
ms_2	51 167		43 92	54 95			48 83	39 82	53 100				
ms_5ms_6					42 98								
n_2	57 97				44 138			48 94					
ob	46 244	55 156	34*156	44 169		44 86	57 87						
pg	49 77	50 73	47 78	58 71		44 83							
rl_1		42 94	44 80	70 93									
v_2	47 67												
v_4	– 72												
bw_1	50 98												

* Chi-square linkage deviation exceeded 0.05 level of probability. † The first number for any given pair is the recombination percentage, and the second number is the population size. All linkage test populations were the F_2 generation. No recombination value is given for the v_4–v_8 test because the two virescent expressions could not be distinguished in the segregating populations.

Table 3. Summary of cotton linkage test results between lines carrying recessive mutants with those carrying dominant mutants.†

	Lc_1	Crp	Lg	L^l	Li	Ms_4	Ms_7	N_1	L♀	R_2
cl_1							56 50			
cu							46 30			
de		60 61			57 51					
fg							40 30			
gl_1		52 82			54 109‡	40 50				
gl_2		49 63			49 200‡	55 80		39 41		
gl_3		65 63			48 200	54 80		24* 41		
st_3	– 95	56 84§	– 95		49 79§	48 93	57 147	– 95	53 95	47 95
ml		36*166			58 89		41 51			
ms_2		53 85§								
ms_5ms_6	× 180‡		× 175‡					× 174‡	× 175‡	× 180‡
st_2	– 139‡		– 139‡					– 139‡	49 194‡	45 195‡
n_2	52 78		12* 78				49 97	– 78	46 79	54 78
ob		43 89§		53 99	48 90§	59 87	51 80			
pg		57 49		60 86	59 74	46 48				
rl_1		62 61		41 54	48 56	45 53				
v_1								38 50		
v_2		40 114§		51 177‡	41 120§	50 170		54 52		
v_3		52 123§		50 97	10*291§					
v_4		52 123§		52 147	41 66§	54 54			47 135	
v_8		54 191‡		48 95	56 208‡	48 75			46 82	

	H_2	Rg	Rd	R_1	Rl_2	Ru	Sm§	Y_1	P_1
cl_1						59 34	43 97		
cu						42 65	47 97		
de			49 78		35*149				
fg						59 97			
gl_1			55 31			44 71	48 97		
gl_2			55 120		45 66	60 43			
gl_3			50 120		52 66	51 43			
st_3	47 95		42 81	46 95	48 86			49 95	54 95
ml			45 119		30* 27				
ms_2					60 122§				
ms_5ms_6	× 180‡		× 180‡					× 180‡	× 180‡
st_2	57 195‡			61 195‡				47 194‡	48 194‡
n_2	63 79			51 79				54 78	46 78
ob			53 151§		35 66§	47 76			
pg			56 82		46 61	43 68			
rl_1					36*160	42 47			
v_1						45 159	54 97		
v_2			48 117§		38 131§	50 127			
v_3			60 123§						
v_4			44 119§		58 71	45 78			
v_8		43 144§	14*339§		44 117§	57 90			

* Chi-square linkage deviation exceeded 0.05 level of probability. † The first number for any given pair is the recombination percentage, and the second number is the population size. Linkage test populations are the backcross generation. ‡ Indicates combined backcross and F_2 generations. § F_2 generations. Recombination values for the pooled backcross and F_2 generation were not calculated for segregation of ms_5ms_6. No direct recombination values could be calculated for segregations of the female steriles st_2 and st_3 with seed character mutants, or the segregation of N_1 and n_2.

Linkage deviations in several other mutant combinations were statistically significant. Some of these were eliminated by tests for chromosome associations; only preliminary information is available for others because testing is difficult and verification will require several years effort. These linkage associations are discussed in the following paragraphs.

The data indicated that both *Crp* and *Ru* were linked with *ml*. The *ml* locus is associated with chromosome 4 (Table 1). Monosome tests have shown *Crp* to be independent of chromosome 4; therefore, the linkage test involving *Crp* and *ml* gave a false indication of linkage. Plants carrying the *Ru* mutant are difficult to work with because of poor seedling emergence, vigor, and seed set. I planted all the seed available to produce the 27 plants that comprised the original linkage population between *ml* and *Ru*. Not enough additional seed has been produced for another linkage test. The F_1 between monosome 4 and *Ru* was obtained, but it was not successfully crossed or self-pollinated. The single F_1 plant involving monosome 4 and *Ru* did not have any unusual phenotypic expression to indicate association. Therefore, the *Ru-ml* linkage will be discounted until resolved by additional testing.

The linkage indicated between *ob* and v_3 is also discounted. Monosome tests have placed *ob* on chro-

mosome 18 and v_3 was independent of this chromosome (Kohel, unpublished).

Similarly, R_2 and $Sm_1{}^S$ cannot be linked. R_2 is associated with chromosome 7 and $Sm_1{}^S$ is independent of that chromosome (J. E. Endrizzi, personal communication).

There were four combinations involving Rl_2 that had linkage values of 35 to 36%. The linkage deviations of Rl_2 with rl_1 and *de* were significant, but deviations of Rl_2 with L^L and *ob* were not. The extreme phenotypic expression of Rl_2 made classification of other mutants in the segregating population difficult. Rl_2 is not linked with L^L because L^L is on chromosome 1 and Rl_2 is independent of this chromosome. The apparent association Rl_2 with *de* was attributed to difficulty in identifying the mutants in the segregating population. The mutants rl_1 and *ob* are especially difficult to separate in segregating populations with Rl_2 because Rl_2 has the round-leaf expression of rl_1 and the open-bud expression of *ob*. Therefore, these linkage associations are discounted here although they are still being tested.

A population of 41 plants indicated the linkage of gl_3 with Ms_7. I am repeating this test, but subsequent linkage tests by E. L. Turcotte (personal communication) did not confirm this association.

The multiple marker line T586 contains Lg, Lc_1, and N_1 seed characters. I measured 12% recombination between Lg in T586 and n_2. However, the segregation of N_1 prevented classification of all plants for n_2 because the latter could only be classified on n_1 n_1 plants. New populations, that do not contain the N_1 mutant, are being synthesized to test linkage of Lg with n_2. Lg is located on chromosome 15, but no monosome 15 is available to test for chromosome association with n_2.

Two years of pooled data indicated no linkage between v_2 and Ru (pooled recombination value was 50.00%), although the first year data indicated that these genes were linked. However, these tests will be repeated because E. L. Turcotte (personal communication) has also reported significant linkage between these two markers. A check of the material used in the crosses and in the field classifications revealed no apparent explanation for this anomalous result. Neither mutant has been associated with a specific chromosome, so the linkage test populations will have to be reconstituted.

Table 4. Summary of cotton linkage test results between lines carrying dominant mutants.†

	$Sm_1{}^S$		Ru		Rl_2		Rd		Ms_7		Ms_4		Li	
Lc_1	56	155	52	76					50	30				
Crp			47	81	50	66	48	123§	56	86	43	97	48	141
Lg	56	155	72	76					57	30				
L?			48	79	35	134‡	57	100			38	97	52	60
Li			54	99	44	90	49	122	53	88	48	109		
Ms_4					48	83	62	71						
Ms_7							54	167						
N_1	55	155	54	76					47	30				
L?	49	156	50	99					53	30				
R_2	38*155		52	87					53	30				
H_2	40	156	50	99					53	30				
Rd			54	108	65	276§								
R_1	50	156	50	99					48	109				
Rl_7			44	88										
Y_1	44	155							47	30				
P_1	50	155	45	87					47	30				

* Chi-square linkage deviation exceeded 0.05 level of probability.
† The first number for any given pair is the recombination percentage, and the second number is the population size. Linkage test populations are the backcross generation. ‡ Indicates combined backcross and F_1 generations. § F_2 generation.

Table 5. Detailed analyses of three populations grown to study Rd-v_8 and Li-v_3 linkages.

Genotype	Segregation (no. plants)				Source	Chi-square analysis x^2			
	A	B	C	Total		A	B	C	Total
V_8-Rd-	85	66	81	232	V_8 vs. v_8	2.63	0.48	0.60	0.01
V_8-rd rd	8	6	9	23	Rd vs. rd	0.29	0.21	0.00	0.01
$v_8 v_8$ Rd-	3	7	13	23	Linkage	44.64	51.84	40.33	135.51
$v_8 v_8$ rd rd	18	21	22	61					
	114	100	125	339	Recombination %	9.92	13.40	18.78	14.22
Li-V_3-	58	71	87	216	Li vs. li	2.35	0.12	0.01	1.10
Li-$v_3 v_3$	4	4	2	10	V_3 vs. v_3	8.22	1.10	0.01	3.99
li li V_3-	9	7	1	17	Linkage	4.48	37.56	102.54	122.75
li li $v_3 v_3$	4	16	28	48					
	75	98	118	291	Recombination %	26.57	12.20	2.42	10.12

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