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Genetic Analysis of Virescent Mutants and the Identification of Virescents v_{12}, v_{13}, v_{14}, v_{15} and $v_{16}v_{17}$ in Upland Cotton[1]

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ABSTRACT

Nine virescent mutants of cotton (*Gossypium hirsutum* L.) were tested for allelism with known virescent mutants and with each other in both greenhouse and field plantings. One mutant was allelic to v_1, two were allelic to yg_1yg_2, and one was allelic to v_{10}. One mutant gave a positive F_1 allelic test with v_2, but segregation in the F_2 established that it was a homoeologue of v_2. Of the remaining mutants, three were identified as simply inherited recessive mutants, and one was a duplicate factor recessive residing on the same chromosomes as v_5 and v_6.

Additional index words: **Inheritance, Linkage, Homoeology, *Gossypium hirsutum* L.**

VIRESCENT mutants were sought for increasing the efficiency of chromosome identification and linkage analysis in Upland cotton, *Gossypium hirsutum* L. Virescents that are expressed in the seedling stage would not have to be grown to maturity for classification. Therefore, many of the studies could be confined to the greenhouse and would not require the time and space of field plantings. Also virescents are valuable for studies of photosynthesis and photosynthetic pigments (Benedict and Kohel, 1968, 1970; Benedict et al., 1972).

We and others previously have identified 11 virescents (Duncan and Pate, 1967; Killough and Horlacher, 1933; Kohel, 1973, 1978; Percival and Kohel, 1974, 1976; Quisenberry and Kohel, 1970; Rhyne, 1955) and a pale green mutant (Murray and Brinkerhoff, 1966) in *G. hirsutum,* and a virescent (Turcotte and Feaster, 1973), and two albivirescents and a light green mutant (Turcotte and Feaster, 1978) in *G. barbadense* L.

Research on the virescents in cotton began with v_1 (Killough and Horlacher, 1933) and yg_1yg_2 (Rhyne, 1955), which express their phenotypes from the seedling to the flowering stage. The next virescent described was v_2, (Duncan and Pate, 1967) called golden crown because its expression is confined to the uppermost leaves of the plant. The expression of v_2 is not only less intense than v_1, but the expression is less reliable.

Both v_1 and v_2 were found in Upland cotton, whereas yg_1yg_2 was found in interspecific hybrids between Upland and Pima, *G. barbadense.* Most Upland cottons are monomeric yg_1Yg_2, a few are dimeric Yg_1Yg_2, and Pima is monomeric Yg_1yg_2. Therefore, most crosses of yg_1yg_2 mutants with normal segregate monogenically, whereas crosses between normal Upland and Pima usually segregate digenically.

The mutant line in *G. barbadense,* Sea Island Virescent, was believed to have been an allele of the Upland v_1 because of the phenotypic similarity. Allelic tests between Sea Island Virescent and a cotton homozygous for v_1v_1 gave virescent F_1 progeny and thus appeared to confirm allelism. However, Turcotte and Feaster (1973) grew F_2 progenies and found that two loci with apparent identical function were involved, and they concluded that the loci were homoeologues. The Sea Island Virescent gene was designated v_7. The combined action of both loci when homozygous, v_1v_1 v_7v_7, results in an extreme virescent, nearly chlorotic expression.

Among the remaining virescents, v_3 and v_9 have extreme expressions with low levels of chlorophyll in affected leaves. The virescent expression is of short duration or even lacking, but plant growth is slower than normal. Plants of the virescent v_{10} and the duplicate factor virescent v_5v_6 have expressions less pronounced than v_2, and frequently the mutant phenotype is not expressed. The mutant plants of v_4 and v_8 are intermediate in expression but greater than v_2. Under conditions favorable for maximum expression, these mutant plants have unique characteristics. The mutant v_4 increases the intensity of virescent expression, but the area immediately adjacent to each gossypol gland in the leaf remains green. Mutant plants of v_8 develop areas on the leaf surface area that have abnormal palisade cell development, and it was called virescent-splash-leaf (Pate and Duncan, 1963).

The objective of the studies reported in this paper were to establish segregation patterns and allelic relationships of nine new virescent mutants.

MATERIALS AND METHODS

Eight of the virescent mutants studied were obtained from researchers at other locations as follows: TA 174 was obtained from J. E. Endrizzi, TA 171, 186, 241, 296, and 297 were obtained from J. E. Quisenberry, TA 240 was obtained from B. Roark, and TA 242 was obtained from R. E. Dilbeck. The ninth, CS 212, was found in our Genetics Nursery.

TA 171 originated in a Paymaster breeding nursery; TA 241 was found in a 'Rilcott 90' cultivar background; TA 296 and 297 were segregants in F_2's of photoperiodic *G. hirsutum* races T191 and 228 × day-neutral Upland (Lubbock Dwarf), respectively; TA 240 was found in a test plot of 'Paymaster 54B'; and CS 212 was found in an F_2 of ms_2 × light brown fibers. The remaining mutants were found in plantings of unknown background.

The mutants were analyzed for allelism and inheritance at College Station, Tex. Inheritance tests were conducted using the Upland standard Texas Marker-1 (Kohel et al., 1970) as a common parent, and certain of the mutants were tested with multiple marker lines. Tests were conducted in the greenhouse in winter and in genetics nursery field plantings. Seeds were planted individually

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Table 1. Tests of allelism among nine new virescent mutants and known virescents in cotton.†

	F_1 expression								
	TA 174	TA 240	TA 296	TA 297	TA 171	TA 186	TA 241	CS 212	TA 242
v_1	-	V	G	G	G	G	G	G	G
v_2	--	G	G	G	G	G	V	G	G
v_3	G	G	G	G	G	G	G	G	G
v_4	G	G	G	G	G	G	G	G	G
v_8	G	G	G	G	G	G	G	G	G
v_9	G	G	G	G	G	G	G	G	G
v_{10}	V	G	--	--	G	G	G	G	G
v_{11}	G	G	--	--	G	G	G	G	G
v_5v_6	-	G	--	--	G	0	G	G	G
yg_1yg_2	--	--	V	V	G	0	-	G	--
TA 174	--	--	--	--	G	--	--	-	-
TA 240	-		-	--	G	G	--	G	--
TA 296	--	--		V	G	0	G	G	G
TA 297	--	-	V		G	0	G	--	G
TA 171	G	G	G	G		G	G	G	0
TA 186	--	G	0	0	G		G	G	0
TA 241	--	--	G	G	G	G		--	--
CS 212	-	G	G	--	G	G	--		0
TA 242	-	--	G	G	0	0	--	0	

† V = virescent, G = green, -- = test not made because of allelic relation established, and 0 = test not made due to results of other tests or number of genes controlling virescent differ.

in expandable peat pellets. Seedlings to be grown in the field plantings were transplanted 3 weeks after seeds were planted.

The descriptions that follow of the virescent mutant plants and their interaction with the environment are based on the observations made at College Station. From discussions with researchers at other locations, it is apparent that the degree and stability of virescent expression varies with locations and seasons.

RESULTS AND DISCUSSION

Results of the tests for allelism are presented in Table 1. Four of the nine mutants were identified as alleles of known virescents. Tests showed that TA 174 is v_{10}, TA 240 is v_1 and TA 296 and 297 are yg_1yg_2. The latter two mutants originated from crosses with *G. hirsutum* race accessions T191 and 228. Because most Upland cottons have the genotype $yg_1yg_1Yg_2Yg_2$, the race stocks must have the genotype $Yg_1Yg_1\ yg_2yg_2$. The latter genotype has not been documented for cultivated Upland stocks but is common in Pima cotton.

TA 241 gave a positive test for allelism in the F_1 test with v_2 plants. However, because Turcotte and Feaster (1973) found that the homoeologues v_1 and v_7 give a positive F_1 allelic test, all suspect or positive F_1 tests for allelisms are verified by extending the test to the F_2. In the F_2 of TA 241 × v_2 lines the population segregated green plants and various levels of virescent expression. The virescent phenotypes varied from the parental phenotype to nearly chlorotic types with extremely low vigor. These results demonstrate the interaction of two independent loci that are apparent homoeologues. Neither v_2 nor the TA 241 gene were found associated with a specific chromosome location.

Five of the nine original virescents were new mutants (Table 2). Genes of the mutants TA 171, 186, 241, and CS 212 segregated as simply inherited recessive factors in backcross and F_2 populations. Because they were shown to be nonallelic (Table 1), they were assigned the gene symbols v_{12}, v_{13}, v_{14}, and v_{15}, respectively.

Genes of the virescent TA 242 segregated as a duplicate recessive (Table 2). The mutant has a weak expression, and segregation was not observed in a second F_2 population.

Table 2. Results of backcross and F_2 tests of inheritance of five new virescent mutants in cotton.

Mutant	Year tested	Green	Virescent	Chi-square†	P
		no.			
TA 171					
		Backcross			
	1974	48	51	0.09	0.95-0.50
	1975	95	85	0.56	0.95-0.50
	1977	95	92	0.05	0.95-0.50
	1978	45	48	0.10	0.95-0.50
	1981	52	41	1.30	0.30-0.20
	Total	335	317	2.10	
	Pooled			0.50	0.50-0.30
	Heterogeneity			1.60	0.95-0.50
		F_2			
	1974	159	51	0.06	0.95-0.50
	1975	87	22	1.35	0.30-0.20
	1977	928	310	0.00	>0.99
	1980	158	37	3.78	0.10-0.05
	1981	148	42	0.85	0.50-0.30
	Total	1480	462	6.04	
	Pooled			1.52	0.30-0.20
	Heterogeneity			4.52	0.50-0.30
TA 186					
		Backcross			
	1975	48	43	0.27	0.95-0.50
	1981	142	136	0.13	0.95-0.50
	Total	190	179	0.40	
	Pooled			0.33	0.95-0.50
	Heterogeneity			0.07	0.95-0.50
		F_2			
	1975	124	42	0.01	0.95-0.50
	1981	72	22	0.13	0.95-0.50
	Total	196	64	0.14	
	Pooled			0.02	0.95-0.50
	Heterogeneity			0.12	0.95-0.50
TA 241					
		Backcross			
	1978	57	39	3.38	0.10-0.05
		F_2			
	1976	73	26	0.08	0.95-0.50
	1977	37	12	0.01	0.95-0.50
	Total	110	38	0.09	
	Pooled			0.04	0.95-0.50
	Heterogeneity			0.05	0.95-0.50
CS 212					
		Backcross			
	1974	69	49	3.39	0.10-0.05
	1981	54	42	1.50	0.30-0.20
	Total	123	91	4.89	
	Pooled			4.78	0.05-0.01
	Heterogeneity			0.11	0.95-0.50
		F_2			
	1974	39	21	3.20	0.10-0.05
	1976	17	8	0.65	0.50-0.30
	Total	56	29	3.85	
	Pooled			3.77	0.10-0.05
	Heterogeneity			0.08	0.95-0.50
TA 242					
		Backcross			
	1980	62	18	0.27	0.95-0.50
		F_2			
	1977	45	5	1.20	0.30-0.20

† Chi-square test 1:1 and 3:1 segregation in backcross and F_2, respectively except for TA 242 which was 3:1 and 15:1 in backcross and F_2, respectively.

Although the allelic test with v_5v_6, a weakly expressed virescent with duplicate factor inheritance, did not indicate allelism, a backcross test was set up to test for linkage with Okra leaf, L^o_2. No recombination has been observed between L^o_2 and v_5 or Laciniate leaf, L^L_1 and v_6 (Kohel, 1973,

Table 3. Backcross linkage analysis of virescent (TA 242) and Okra leaf in cotton [(Okra leaf-Green × normal leaf-virescent) × normal leaf-virescent].

		Chi-square analysis and recombination†		
Phenotype	Plants	Source		Chi-square
Okra leaf-green	39	Okra vs. normal	=	0.80
Okra leaf-virescent	5	Green vs. virescent	=	0.27
Normal leaf-green	23	Linkage	=	6.67
Normal leaf-virescent	13	Recombination	=	24.09%
Total	80			

† Recombination calculated by formulae for duplicate factor recessive linked to single factor dominant (Allard, 1956).

unpublished). In the backcross population genes of the virescent TA 242 segregated as a duplicate factor recessive and was linked to L^o_2 with a recombination value of 24.09% (Table 3). Virescent TA 242 was not tested with L^L_1. Therefore, TA 242 represents a second virescent duplicate pair in these two linkage groups. TA 242 is assigned the genotype $v_{16}v_{16}v_{17}v_{17}$.

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