THE INHERITANCE AND LINKAGE RELATIONS OF CURLY LEAF AND VIRESCENT BUD, TWO MUTANTS IN ASIATIC COTTON

By CHI PAO YU

Agricultural College, National Central University, Chungking, China

CONTENTS

I. Introduction

In a previous paper (1939) the writer mentioned that since selfing was used in cotton breeding in China, many recessive genes have been discovered. Here two new mutants are reported, curly leaf, **cu**, and virescent bud, $\mathbf{v}_1$.

The curly mutation described in this paper was discovered by Mr Kuo, H.C., in 1928. The writer recently found a similar character in another variety, but whether it is the same as **cu** has not yet been determined. Mr Hsi found a similar form in Upland cotton in a breeding field of the Central Institute of Cotton Improvement in China. Although both the "Crinkled leaf" of Harland (1918) in New World cotton, and the "Crumpled" of Hutchinson (1932*a*) in Old World cotton were characters affecting leaf form, they do not seem to be related to the present mutation.

The writer obtained four kinds of virescent, but each of them has its special expression. Two of them have been identified and proved to be controlled by two unrelated factors. The $\mathbf{v}_1$, here reported, is one of the two. In Upland cotton there was a virescent gene discovered by Killough & Horlacher (1933), who concluded that it was a simple Mendelian factor, recessive to the normal green and inherited independently of anthocyanin pigmentation.

In this paper, in addition to studying the inheritance of the two factors, special attention is paid to testing the correlations between these two and other characters, such as anthocyanin pigmentation, leaf shape, corolla colour, lint colour and seed fuzz. The inheritance of these characters was separately studied by Leake (1911), Kottur (1923), Feng (1926), Hutchinson (1931, 1932*b*, 1934, 1935), Yu & Hsi (1934) and Yu (1939).

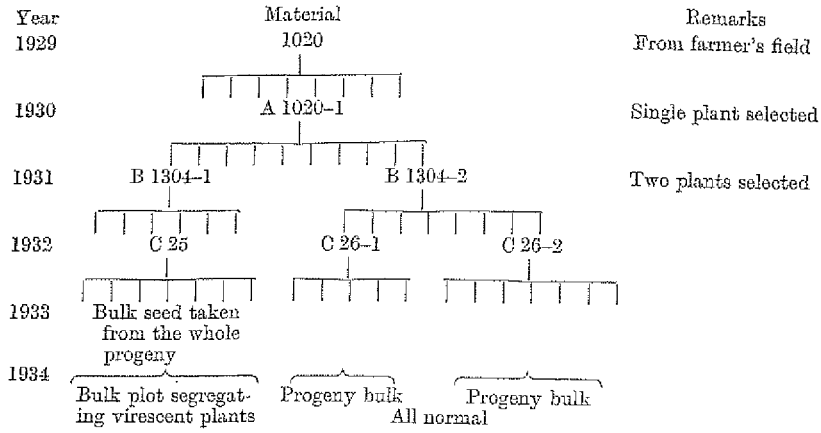
II. Description of the mutants

Curly leaf, **cu**, was first found as a single mutant in an increase field of an improved variety known as "Small White Flower". The mutant does not differ from normal in height. The upper surface of the leaf seems to be covered with a layer of waxy substance, and thus appears very smooth and glabrous. The colour of the leaf is much deeper than that of the normal type. Its peculiarity is that the margin of the leaf curls upwards and inwards. The mutant cannot easily be distinguished from normal until one week after germination, when its curly character distinctly appears on the cotyledons. The true leaf expresses its peculiarity at a very young stage. The petals of the curly plant are only about one-half the size of those of the normal. Its boll and seed are also smaller than those of the normal type. The germination power of the mutant seeds is also below normal, especially under bad environmental conditions.

The peculiarity of the virescent mutation is that the bud and enfolding leaves of the mutant are always yellowish green in colour. Its cotyledons are virescent when first expanding, gradually changing to normal green as the true leaves, which are virescent, appear. As the terminal bud extends upward, it remains virescent, but the lower maturing leaves turn green. There are always virescent young leaves at the top of the mutant plant so long as it lives. A virescent plant produces only a few fruits, possibly because it possesses less pigment for photosynthesis.

The virescent plant was first found in 1934 in a selfed strain. The particular strain was the offspring of a single boll collected in 1929, and selfed every generation thereafter. The original boll, the ancestor of the mutant, produced normal plants. The 1930 and 1931 crops were grown from single plant selections. Two plants were selected in the 1931 crop giving two boll rows in 1932. In the progeny of B 1304–2, two plants were selected from which further generations were grown in 1933 and 1934, and gave normal plants only. In the progeny of B 1304–1, one plant (C 25) was selected. Virescent appeared in a plot grown from mixed seed

of the offspring of C 25, showing that the mutation must have occurred in C 25 or its progeny. The following diagram shows their pedigree:



III. MATERIAL AND METHODS

The following table shows the material used in this study:

Characteristics	Sources
Curly leaf, green plant, broad-lobed leaf, non-virescent, white lint, tufted seeds	Mutant strain
Non-curly leaf, sun red plant, virescent bud, fuzzy seed	Mutant strain
Heterozygous virescent	Segregating population
Non-curly, narrow-lobed leaf, brown lint	Selfed strain

Since both curly leaf and virescent are seedling characters, the seeds were sown in sand instead of ordinary soil in order to get better germination.

The virescent bud accidentally appeared in two rows of the breeding plot in great numbers. When mutants were found in these two rows, the reserve seed was sown. It was not expected that a good segregating ratio would be obtained. The plants of the segregating population were selfed, and at the same time they were crossed with virescent individuals. After harvesting, the genotype of the individuals in the population was tested by using the selfed seeds. There were homozygous and heterozygous virescent types, and the cross made between the virescent and the latter type was considered as a backcross.

In this paper, the important portion of the segregating population was classified for leaf shape by use of Hutchinson's (1934) mean index, but other portions were classified by eye. However, when the classification of any individual by eye was difficult, the index was used as a check.

IV. Experimental results

A. *Curly leaf*

(1) *Inheritance of curly leaf.* Reciprocal crosses between curly leaf and normal gave F_1's with normal leaf, the curly being completely recessive. Classifications of F_2 and backcross populations are included in Table I.

TABLE I

Family	Generation	Non-curly **Cu**	Curly leaf **cu**
34	F_2	319	98
38	F_2	892	274
159	F_2	276	90
161	F_2	236	78
805	F_2	353	123
821	F_2	245	79
1910	F_2	259	91
2279	F_2	136	54
2679	F_2	152	58
	Total	2868	945
Exp. (3 : 1)		2859·75	953·25
67–70	Backcross	13	13
Exp. (1 : 1)		13	13

Selfing normal F_2 plants gave the following results:

Family	Segregating plant **Cucu**	Homozygotes **CuCu**
159	30	17
161	31	14
Total	61	31
Exp. (2 : 1)	61·3	30·7

From the above figures, it is evident that curly leaf, **cu**, is a simple Mendelian recessive.

(2) *Linkage between curly leaf and leaf shape.* Crossing plants with non-curly, narrow-lobed leaf, with plants with curly, broad-lobed leaf, the F_1 was non-curly and intermediate in leaf shape. The F_1 plants were selfed and crossed to the double recessive. Distributions of F_2 and backcross progenies are given in Table II.

From the distribution of the mean index of the F_2's it is clear that the value 3·0 can be used to separate the whole population into two classes: values larger than 3·0 belong to the narrow-lobed and intermediate class, and those smaller than 3·0 belong to the broad-lobed class. Frequency arrays of progenies of two F_2 plants with mean index 3·0 are included in Table II, and it is clear that they were homozygous broad. From this classification, the distribution of the backcross may be summarized as follows:

Family	Non-curly, narrow **CuL**	Non-curly, broad **Cul**	Curly, narrow **cuL**	Curly, broad **cul**
67–70	12	1	1	12

These figures clearly show that narrow-lobed and normal leaf, or broad-lobed and curly are linked pairs. However, the population was too small to determine the cross-over value.

TABLE II

Constitution	Phenotype	2·0	2·2	2·4	2·6	2·8	3·0	3·2	3·4	3·6	3·8	4·0	4·2
P_1 cul	cul	.	.	4	11	3	1	.	.	.	.	.	.
P_1' CuL	CuL	.	.	.	.	.	.	.	.	.	.	1	1
F_1 CuLcul	CuL	.	.	.	.	.	.	.	1	8	6	8	2
Backcross to cul	Cu	.	.	.	1	.	.	.	1	4	3	4	.
	cu	.	.	1	3	6	2	.	.	.	1	.	.
F_2 (1936)	Cu	.	.	6	29	16	12	4	18	37	75	82	76
	cu	.	3	42	49	32	3	5	13	12	4	7	2
F_2 (1937)	Cu	.	.	5	8	14	1	9	26	45	42	47	37
	cu	1	1	7	36	15	2	3	3	10	7	5	5
F_3 (from F_2, mean index = 2·97)	Cu	.	.	.	5	7	1	.	.	.	.	.	.
F_3 (from F_2, mean index = 2·98)	Cu	.	.	2	.	1	.	.	.	.	.	.	.

Constitution	Phenotype	4·4	4·6	4·8	5·0	5·2	5·4	5·6	5·8	6·0	6·2	6·4	6·6
P_1 cul	cul	.	.	.	.	.	.	.	.	.	.	.	.
P_1' CuL	CuL	.	3	2	2	3	2	6	1	2	.	1	.
F_1 CuLcul	CuL	1	1	1	.	.	.	.	.	.	.	.	.
Backcross to cul	Cu	.	.	.	.	.	.	.	.	.	.	.	.
	cu	.	.	.	.	.	.	.	.	.	.	.	.
F_2 (1936)	Cu	31	25	22	25	23	29	26	12	7	3	2	1
	cu	3	1	.	.	1	.	.	.	.	.	.	.
F_2 (1937)	Cu	24	15	19	14	13	4	5	2	.	.	1	.
	cu	.	.	1	.	.	.	1	.	.	.	.	.
F_3 (from F_2, mean index = 2·97)	Cu	.	.	.	.	.	.	.	.	.	.	.	.
F_3 (from F_2, mean index = 2·98)	Cu	.	.	.	.	.	.	.	.	.	.	.	.

Classifying the distributions of the F_2 progenies in the same way gave:

Family and year	Non-curly, narrow CuL	Non-curly, broad Cul	Curly, narrow cuL	Curly, broad cul
34–39 (1936)	498	63	48	129
34–39 (1937)	303	28	35	62
Total	801	91	83	191
Exp. (cross-over value = 16·6 %)	785·8	88·7	88·7	202·8

F_3 progenies were grown from some F_2 plants in each class. The double recessive class all bred true. Among the other three groups some bred true, some segregated. The results are given in Table III.

According to the above data, it is plain that curly leaf and leaf shape belong to the same linkage group and that their cross-over value is not far from 16·6 %.

TABLE III

F_2 Phenotype	F_2 Genotype		Observed	Expected (16·6 % crossing over)
CuL	**CuCuLL**		30	29·2
	CuCuLl		9	11·6
	CucuLL		13	11·6
	CucuLl		61	60·6
		Total	113	$0{\cdot}90 > P > 0{\cdot}80$
Cul	**CuCull**		3	2·3
	Cucull		22	22·7
		Total	25	$0{\cdot}70 > P > 0{\cdot}50$
cuL	**cucuLL**		3	1·4
	cucuLl		12	13·6
		Total	15	$0{\cdot}30 > P > 0{\cdot}10$

(3) *Free assortment between curly leaf and other characters.* Segregations showing independent assortment between curly and four characters other than leaf shape are summarized below:

TABLE IV

Characters	Genes*	No. of families	XY	Xy	xY	xy	χ^2	P
Curly: anthocyanin	**Cu R_2**	2 F_2 Coupling	454 (450)	144 (150)	153 (150)	49 (50)		
Curly: corolla colour	**Cu Y_2**	3 F_2 Coupling	653 (618)	203 (206)	187 (206)	56 (69)	6·2	0·1
Curly: light brown lint	**Cu?**	1 F_2 Coupling	257 (250·5)	77 (83·5)	50 (45)	10 (15)†	2·9	0·2–0·3
		1 B.C. Coupling	8 (6)	4 (6)	8 (6)	4 (6)		
Curly: seed fuzz	**Cu F_2**	1 F_2 Coupling	228 (221·6)	70 (73·9)	77 (73·9)	19 (24·6)		

* Symbols according to Hutchinson and Silow's revised nomenclature (personal communication).

† Deficiency of curly plants sown for lint colour, on account of poorer fruiting. Expectation calculated on basis of normal : curly actually observed (3 : 1, 3 : 1).

B. *Virescent bud*

(1) *Inheritance of virescent bud.* The virescent mutant was crossed with normal. The F_1 was indistinguishable from normal. F_2 and backcross data are shown in Table V.

TABLE V

Family	Generation		Non-virescent, V_1	Virescent, v_1
805 (1935)	F_2		841	285
805 (1937)	F_2		366	110
821	F_2		250	74
		Total	1457	469
Exp. (3 : 1)			1444·5	481·5
22–4—22–25	Backcross		36	39
23–1—23–27	Backcross		63	42
		Total	99	81
Exp. (1 : 1)			90	90

Testing normal F_2 plants the following results were obtained:

Family	Homozygous normal V_1V_1	Segregating V_1v_1
22–3—23–29	11	25
Gh 1—Gh 71	20	31
Total	31	56
Exp. (1 : 2)	29	58

From the above facts, it is plain that the virescent character is due to a single Mendelian recessive.

(2) *Correlations between virescent and other characters.* Two-factor segregations involving virescent and four other characters have been investigated, and all showed independent assortment. No linkage involving virescent has yet been observed. Data are summarized in Table VI.

TABLE VI

Characters	Genes	No. of families	XY	Xy	xY	xy
Virescent: anthocyanin	V_1R_2	2 F_2 Repulsion	1118 (1083·4)	342 (361·1)	339 (361·1)	127 (120·4)
Virescent: corolla colour	V_1Y_a	1 F_2 Repulsion	229 (221·6)	79 (73·9)	64 (73·9)	22 (24·6)
Virescent: curly	V_1Cu	2 F_2 Repulsion	468 (450)	148 (150)	130 (150)	54 (50)
Virescent: seed fuzz	V_1F_z	1 F_2 Coupling	233 (221·6)	75 (73·9)	65 (73·9)	21 (24·9)

V. DISCUSSION

Mutations phenotypically similar to the two types described in this paper have been discovered more than once, but their homology with these two standard types has not been established. There is therefore no information on the mutability of the curly and virescent loci.

The cross-over value of 16·6 % between leaf shape and curly has been established. Although Hutchinson (1934) found that leaf shape and lint colour were linked, the writer found that curly and lint colour were independently inherited. However, the lint colour investigated by Hutchinson was a dark brown showing full dominance (personal communication), whilst that used in this study gave an intermediate heterozygote. The lint colour gene used in this investigation was tested for linkage with leaf shape and in F_2 gave the following figures, which show free assortment:

Family	Generation	Narrow brown	Narrow white	Broad brown	Broad white
2505	F_2	348	120	112	31
Exp. (9:3:3:1)		343·6	114·6	114·6	38·2

It is evident that the lint colour gene used in this investigation was different from that which has been shown by Hutchinson to be linked with leaf shape, and would therefore be expected to segregate independently of curly.

VI. Summary

1. Curly leaf, cu, and virescent bud, v_1, are two newly discovered genes affecting seedlings. Both of them are completely recessive, and form single-factor pairs with the normal.

2. It is shown that curly leaf and leaf shape are linked, with a cross-over value not far from 16·6 %.

3. Curly leaf and the following characters are independently inherited: anthocyanin pigmentation, corolla colour, lint colour and seed fuzz.

4. Virescent bud and the following characters are independently inherited: curly leaf, anthocyanin pigmentation, corolla colour, seed fuzz, leaf shape and yellow seedling.

5. From the linkage studies of Hutchinson and the writer, it is concluded that the lint colour genes which they investigated are distinct.

VII. Acknowledgement

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