

## THE GENETICS OF BLACKARM RESISTANCE

XI. *GOSSYPIUM ANOMALUM*

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## PREVIOUS WORK

Six genes, each of which directly confers resistance to blackarm disease (*Xanthomonas malvacearum*) in cotton, have so far been identified. All of these are either fully or partially dominant. A further gene, **B**<sub>6m</sub>, has been found, and this acts as a powerful intensifier of resistance, though in the absence of a major resistance gene it has no demonstrable effect. Details of this work are given in previous papers of this series.

The system of blackarm grading used in this paper is that described and illustrated by Knight (1944): briefly, '0' represents complete absence of the disease and '12' the full attack to be found on the leaves of 'Egyptian' cotton varieties after they have been sprayed with inoculum. The method of making the inoculum and of spraying the plants with it were described by Knight (1946).

## DESCRIPTION OF STRAINS

*Gossypium anomalum* (*n*=13) is a wild species belonging to the *B* genom. It grows in South-West Africa and along the southern fringes of the Sahara, almost from the Atlantic to the Red Sea. In the Sudan it is found in a broad belt between the 5 and 20 in. isohyets. The northern, more arid, fringe of this belt runs from *jebel* (rocky hill) top to *jebel* top across the northern parts of Khartoum, Kordofan and Darfur provinces. Southwards, with increasing rainfall, *G. anomalum* can be found on fixed low dunes, or ridges, of sand or grit, and still further south on mature sand plains (Knight, 1949*a*). A detailed description of the species is given by Hutchinson, Silow & Stephens (1947).

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The strain of *anomalum* used as parent in the crosses described in this paper was that obtained by Chevalier (1933), now maintained in the Empire Cotton Growing Corporation's Type Collection at Shambat under the number WB 1-1. Fifteen accessions of *anomalum* from widely different localities are represented in the Type Collection: they show so little variation (Knight, 1949*a*) that it is probable that the genetic analysis of blackarm resistance made for Chevalier's accession applies equally to all.

*G. arboreum* ( $n=13$ ; *A* genom). The blackarm susceptible parent used in the crosses described in this paper was an *arboreum* from Java, chosen from amongst a large number of strains in the Type Collection as being fully susceptible to blackarm. Its registered number in the Collection is ANCJ. 2.

TRANSFERENCE OF RESISTANCE FROM *GOSYPIUM ANOMALUM* TO *G. ARBOREUM* (JAVA)

$F_1$  of *G. anomalum*  $\times$  *Java*

Two  $F_1$  families of *G. anomalum*  $\times$  *Java* were grown in 1946. In the first, BA 704/46, *Java* was the female parent; this family comprised thirty-one plants. The reciprocal hybrid, BA 705/46, consisted of four plants. These thirty-five plants showed blackarm symptoms ranging from grade '9' to '10' compared with a range from '8' to '10' in the neighbouring *Java* control. Only one plant survived in the *anomalum* control and this was graded as '3'.

First backcross to *Java* and subsequent selfed generations

Two plants in an early (out-of-season) sowing of BA 704/46 and one in BA 705/46 were used as female parents in backcrossing to *Java*. The crosses with BA 704/46 gave three plants and those with BA 705/46 gave six plants. These plants were all individually selfed, but only two yielded viable seed (both deriving from the progeny of BA 705/46). The blackarm grading of these two first backcross  $F_2$  progenies is given in Table 1.

Table 1. Blackarm grading of  $F_2$  of first backcross to *Java*

Family no.	Blackarm grade									Totals		Ratio
	'2'	'3'	'4'	'5'	'6'	'7'	'8'	'9'	'10'	Rest.	Sus.	
BA 372/47	—	—	—	2	—	—	7	7	4	2	18	1:9:0
BA 375/47	—	—	—	1	—	—	14	3	5	1	22	1:22:0
Totals	—	—	—	3	—	—	21	10	9	3	40	1:13:3
Controls:												
<i>G. anomalum</i>	3	3	3	—	—	—	—	—	—	—	—	
<i>Java</i>	—	—	—	—	—	—	8	14	—	—	—	

Two of the three resistant plants shown in Table 1 proved fertile on selfing: both yielded progenies consisting of resistant plants, though both progenies overlapped the *Java* control which in that season showed a range of '7' to '9' as compared with its normal range of '8' to '10'. A number of the susceptible plants shown in Table 1 were also selfed. These yielded seventeen progenies (omitting those of less than five plants), and these are classified for blackarm grade in Table 2.

The ten resistant plants in BA 307/48 (Table 2) were selfed: three of them proved reasonably fertile and these gave the progenies classified in Table 3.

It is clear, from the distribution in BA 96/49 (Table 3) that the parent plant did not

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carry any major resistance gene. This family (BA 96/49) derived from the single grade '6' plant in BA 307/48 (Table 2). This plant therefore belongs, genotypically, with the susceptible, and the family thus contained a total of nine resistant plants and seventy-three susceptibles: a ratio of 1:8.1.

Table 2. *Blackarm grading of F<sub>3</sub> of first backcross to Java*  
(Progenies deriving from parents of grade '8'-'10'.)

Family no.	Blackarm grade						
	'4'	'5'	'6'	'7'	'8'	'9'	'10'
BA 305/48	—	—	—	1	3	1	—
BA 306/48	—	—	—	—	3	1	—
BA 307/48	1	8	1	13	28	18	13
BA 308/48	—	—	—	—	—	6	19
BA 309/48	—	—	—	—	1	3	17
BA 313/48	—	—	—	—	7	13	14
BA 318/48	—	—	1	3	4	3	6
BA 319/48	—	—	—	1	8	32	68
BA 320/48	—	—	—	—	—	1	4
BA 321/48	—	—	—	—	1	4	6
BA 322/48	—	—	—	3	3	1	—
BA 323/48	—	—	—	1	2	2	—
BA 324/48	—	—	—	2	2	7	5
BA 325/48	—	—	1	1	11	8	8
BA 326/48	—	—	—	1	2	5	3
Java	—	—	—	8	29	3	—

Table 3. *Blackarm grading of F<sub>4</sub> of first backcross to Java*

Family no.	Parent grade	Blackarm grade									
		'1'	'2'	'3'	'4'	'5'	'6'	'7'	'8'	'9'	'10'
BA 92/49	'5'	2	7	—	2	3	—	—	—	—	—
BA 94/49	'5'	20	14	7	10	14	8	—	—	—	—
BA 96/49	'6'	—	—	—	—	—	7	23	24	1	—
Java	—	—	—	—	—	—	—	12	26	—	—

Table 4. *Blackarm grading of F<sub>2</sub> of second backcross to Java*

Family no.	Parent grade	Blackarm grade									Totals		Ratios
		'2'	'3'	'4'	'5'	'6'	'7'	'8'	'9'	'10'	Rest.	Sus.	
BA 114/51	'6'	—	—	—	3	—	—	6	57	12	3	75	1:25.0
BA 115/51	'6'	—	—	1	3	—	—	4	56	15	4	75	1:18.8
BA 116/51	'6'	—	—	3	7	—	—	5	27	12	10	44	1: 4.4
BA 117/51	'8'	—	1	5	6	1	—	16	43	13	13	72	1: 5.5
BA 118/51	'8'	—	—	4	3	—	—	27	44	5	7	76	1:10.9
Totals	—	—	1	13	22	1	—	58	227	57	37	342	1: 9.2
Controls:													
<i>anomalum</i>	—	—	4	7	11	1	—	—	—	—	23	—	—
Java	—	—	—	—	—	—	—	2	24	13	—	39	—

*Second backcross to Java and its F<sub>2</sub>*

Within BA 94/49 (Table 3), five plants of grade '1' and five of grade '5' were chosen for crossing with Java. All ten of these progenies consisted of plants showing a similar degree of susceptibility to Java. Within one of the progenies deriving from the cross of a grade '1' parent with Java, three grade '6' plants and two grade '8' plants were selfed. The classification of the progenies of these plants is shown in Table 4.

*Third backcross to Java and its F<sub>2</sub>*

In family BA 117/51 (Table 4) the five grade '4' plants were crossed with Java, the latter being the female parent. Two of these plants proved infertile, but three progenies, of seven, thirteen and seven plants respectively, were raised from the other crosses. All twenty-seven of these third backcross  $F_1$  plants showed grade '9'-'10' blackarm attack, compared with Java, which showed a range from '8' to '10' and *G. anomalum*, which showed a range from '3' to '5'. BAR 21/1, a homozygous resistant line selfed out from the first backcross to Java and now in  $F_7$ , showed symptoms ranging from '2' to '5'.

Twelve of these susceptible third backcross  $F_1$  plants were selfed. Their progenies, grown in 1953-4 season, were classified for blackarm resistance into the distributions shown in Table 5.

Table 5. *Blackarm grading of F<sub>2</sub> of third backcross to Java*

Family no.	Blackarm grade									Totals		Ratios
	'2'	'3'	'4'	'5'	'6'	'7'	'8'	'9'	'10'	Rest.	Sus.	
BA 316/53	—	—	3	9	4	2	16	45	40	18	101	1:5.6
BA 317/53	—	3	6	4	3	1	18	40	60	17	118	1:6.9
BA 318/53	—	—	2	5	6	1	24	37	53	14	114	1:8.1
BA 319/53	—	—	1	1	2	2	12	19	16	6	47	1:7.8
BA 320/53	—	3	15	11	3	—	10	124	63	32	197	1:6.2
BA 321/53	—	3	13	5	1	—	4	51	68	22	123	1:5.6
BA 322/53	—	6	16	11	1	—	3	60	83	34	146	1:4.3
BA 323/53	—	5	19	5	—	—	4	101	86	29	191	1:6.6
BA 324/53	—	3	1	1	2	—	1	11	17	7	29	1:4.1
BA 325/53	—	1	8	5	3	—	—	81	83	17	164	1:9.6
BA 326/53	—	3	13	11	2	—	2	59	101	29	162	1:5.6
BA 327/53	—	1	4	7	3	—	1	48	92	15	141	1:9.4
Totals	—	28	101	75	30	6	95	676	762	240	1533	1:6.4
Controls:												
<i>anomalum</i> 'a'	2	11	2	—	—	—	—	—	—	15	—	—
<i>anomalum</i> 'b'	—	2	30	5	—	—	—	—	—	37	—	—
BAR 21/2 'b'	—	—	2	16	4	—	—	—	—	22	—	—
Java 'a'	—	—	—	—	—	—	3	6	7	—	16	—
Java 'b'	—	—	—	—	—	—	1	25	15	—	41	—

Note. The 'a' controls were adjacent to families BA 316-319/53; the 'b' controls were adjacent to BA 320-327/53. BAR 21/2 is a homozygous resistant line selfed out from the second backcross to Java.

## DISCUSSION

*The nature of resistance in Gossypium anomalum*

The xerophytic ecology and slow germination of *G. anomalum* almost exclude the possibility of attack by *Xanthomonas malvacearum* so that it is most improbable that blackarm disease has had any selective impact on this species—or, for similar reasons, on any other truly wild species of *Gossypium*. For these reasons Knight (1948*a*) regarded resistance, where it occurs in the wild *Gossypiums*, as being preadaptational, and hence he inferred that it would prove to be genetically simple in its inheritance.

The crosses outlined in this paper show that *anomalum* resistance is recessive, but the ratios obtained on selfing the first backcross to Java are far from the 3:1 of expectation on a monofactorial basis. Such divergence from expectation is, however, not uncommon in crosses between widely divergent species (Silow, 1941; Knight, 1948*b*) and the gap between observation and expectation narrows as one or other parental background is

re-established by further backcrossing. In the *anomalum* crosses, the ratio of susceptible:resistant plants fell from 13.3:1 in the  $F_2$  of the first backcross (Table 1) to 9.2:1 and 6.4:1 in the  $F_2$ 's of the second and third backcrosses respectively (Tables 4 and 5), two of the families in the third backcross  $F_2$  giving ratios of approximately 4:1. These figures, taken in conjunction with the linkage data given below, leave no doubt that a single recessive gene is involved. This gene has been called  $b_8$ . No homology tests have yet been made between it and the other seven known resistance genes, but its transference to Sakel has been initiated, via the hexaploid *G. anomaldense* and this transference will in due course render such gene homology tests possible. Meanwhile, the behaviour of  $b_8$  is so different from that of the other seven genes that it seems unlikely that it will prove allelic to any of them.

A comparison of the resistance grade of *G. anomalum* with that conferred by  $b_8$  (Tables 4 and 5) shows the  $b_8 b_8$  material to be rather less resistant than the parental *anomalum*. This difference is probably due to 'ancillary' resistance, brought about by the response of *anomalum* to the selective effect of a xerophytic environment, causing it to acquire a capacity for rapid hardening of its leaves under conditions of lowered humidity.

#### *Linkage between $b_8$ and the $R_2$ locus*

It was shown by Silow (1941) that the large petal spot, which is always present in *G. anomalum*, is due to the interaction of two genes  $R_2^{OS}$  and  $R_3^{GO}$ . Java also has a pronounced petal spot due to the presence of  $R_2^{AS}$ , the  $R_3$  locus in Java being represented by the  $r_3^{OO}$  allele. Thus *G. anomalum* is of  $b_8 b_8 R_2^{OS} R_2^{OS} R_3^{GO} R_3^{GO}$  genotype and Java is  $B_8 B_8 R_2^{AS} R_2^{AS} r_3^{OO} r_3^{OO}$ . *G. arboreum* cottons of  $R_2^{OS} R_2^{OS} r_3^{OO} r_3^{OO}$  genotype have 'ghost' spot, that is the petal spot is replaced by a white area (pl. 18, fig. 3, in Silow's paper), and Silow obtained such 'ghost' segregates in crosses between *G. anomalum* and *G. arboreum*.

In the present work it was noted that homozygous resistant lines, selfed out from the first backcross of *anomalum* to Java, were of the 'ghost' type, that is their genotype was  $b_8 b_8 R_2^{OS} R_2^{OS} r_3^{OO} r_3^{OO}$ . An examination of all plants which survived to flowering stage in family BA 327/53 (Table 5) showed evidence of strong linkage between  $R_2^{OS}$  and  $b_8$  (Table 6).

Table 6. *Classification of plants in BA 327/53 for blackarm resistance and petal spot*

	Resistant	Susceptible
Ghost spot	14	2
Spot	—	135

Petal spot data were collected only from families BA 320–327/53 (Table 5) in the third backcross  $F_2$ , because in this group the distinction between 'resistant' and 'susceptible' allowed of no confusion due to overlapping, resistant plants being of grade '6' or under and susceptibles being of grade '8' or over. An examination of all resistant plants which reached flowering stage in these progenies (including BA 327/53 listed above) showed there were 175 with ghost spot and 5 with spot. An examination of all susceptible plants which had flowered at thinning time showed 589 with a petal spot and four with ghost spot.

An accurate estimate of the crossover value of  $R_2^{OS}/b_8$  will become possible when normal 3:1 ratios of susceptible to resistant plants are obtained in later backcross  $F_2$ 's.

Meanwhile, a rough estimate can be obtained from the totals for the 'resistant' group of plants. The genotypes expected in  $F_2$  with free assortment are:

1	$R_2^{AS} R_2^{AS} B_8 B_8$	Spot	Susceptible
2	$R_2^{AS} R_2^{OS} B_8 B_8$	Spot	Susceptible
2	$R_2^{AS} R_2^{AS} B_8 b_8$	Spot	Susceptible
4	$R_2^{AS} R_2^{OS} B_8 b_8$	Spot	Susceptible
1	$R_2^{OS} R_2^{OS} B_8 B_8$	Ghost spot	Susceptible
2	$R_2^{OS} R_2^{OS} B_8 b_8$	Ghost spot	Susceptible
1	$R_2^{AS} R_2^{AS} b_8 b_8$	Spot	Resistant
2	$R_2^{AS} R_2^{OS} b_8 b_8$	Spot	Resistant
1	$R_2^{OS} R_2^{OS} b_8 b_8$	Ghost spot	Resistant

Thus the normal expectation would be 9 spot-susceptible:3 ghost-susceptible:3 spot-resistant:1 ghost-resistant. With free assortment, the 180 resistant plants would show an expectation of 135 spot:45 ghost, and the observed ratio of 5 spot:175 ghost suggests a crossover value of about 1.4%.

The proof that  $b_8$  and the  $R_2$  locus are linked extends the knowledge which is accumulating with regard to the  $R_2$  chromosome both in Asiatic ( $A$  genom) and New World ( $AD$  tetraploid) species. The known linkage groups in this chromosome are now:

Genes	Crossover Old World	Value New World	Reference
$Ch1_2 - R_2$	9	—	Feng, 1926
$Cl_2 - R_2$	30	10.4	Patel, Munshi & Patel, 1947; Silow, 1946
Wilt resistance - $R_2$	?	—	Ramiah & Paranjape, 1947
$b_8 - R_2$	1.4	—	—

In addition it seems likely that the  $H_1$  locus is also on this chromosome (Knight, 1954). Silow's (1946) view as to the homology of the  $R_1$  chromosome, in the  $D$  genom in New World cottons, with the  $R_2$  chromosome in Asiatic ( $A$  genom) cottons, would suggest the probability of a similar series of linkage groups in both chromosomes. Several of the wild diploid New World *Gossypiums* are resistant to blackarm, and this resistance is recessive (Knight 1948*a*, 1949*b*). This raises the possibility of a recessive gene, homologous in origin with  $b_8$ , which might be expected to be linked with  $R_1$ . To test this hypothesis it will be necessary to transfer an  $R_1$  gene from a resistant New World wild *Gossypium* to a spotless, blackarm-susceptible, cultivated New World amphidiploid. A proportion of such transferred  $R_1$  'spot' types, when selfed out in the homozygous phase, would be likely on this theory to be blackarm resistant. Work on these lines has been initiated at Shambat.

#### SUMMARY

Resistance to blackarm disease (*Xanthomonas malvacearum*) in *Gossypium anomalum* ( $n=13$ ;  $B$  genom) is controlled by a single recessive gene,  $b_8$ . This gene has been transferred to *G. arboreum* where it confers resistance rather less than that typical of *G. anomalum*. The extra resistance of *G. anomalum* is presumed to be due to factors for rapid leaf hardening evolved in response to the requirements of the xerophytic environment of this species. The gene  $b_8$  is closely linked with  $R_2^{OS}$  in *G. anomalum*.

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