Genet. Res., Camb. (1963), **4**, *pp.* 382–388
Printed in Great Britain

The genetics of bacterial blight resistance in cotton

FURTHER EVIDENCE ON THE GENE B_{6m}

By J. H. SAUNDERS and N. L. INNES

Agricultural Research Division, Department of Agriculture, Khartoum, Republic of the Sudan, and Empire Cotton Growing Corporation

(*Received* 8 *March* 1963)

1. INTRODUCTION

Resistance to bacterial blight (blackarm) disease of cotton (*Xanthomonas malvacearum* (E. F. Sm.) Dowson) is caused by a series of genes isolated and described by Knight (1957). On the basis of the degree of leaf infection after inoculation Knight graded the resistance level from grade '0', which represents complete immunity, to grade '12' which represents complete susceptibility. Grade '11' has since been omitted from the scale.

Knight (1953) presented evidence for an important modifying gene B_{6m} which he transferred from diploid *Gossypium arboreum* to the tetraploid commercial cotton, Sakel (*G. barbadense*). In a 'natural' progeny of a second Sakel backcross during the transference from *G. arboreum* he observed grade '3' resistance in certain plants. This was near immunity to bacterial blight and further tests revealed that these plants contained not only the resistance gene B_2, coming from Sakel B_2B_2 derivatives, but also an unknown resistance factor, which was named B_{6m} and was presumed to have come from *G. arboreum*. Knight later confirmed its origin from this species by transfer tests. All later work with B_{6m} was integrated into a breeding programme designed to produce varieties with field immunity to bacterial blight, i.e. it was added to strains already carrying the genes B_2 or B_2B_3 in a homozygous condition. The greatly enhanced resistance thus obtained led Knight to believe that B_{6m} was a modifier and conferred no resistance alone. However, B_{6m} had not been isolated by itself on a Sakel background during this programme. The requirements of later programmes made it necessary to have available Sakel stocks homozygous for B_{6m} only. This work is described here and it will be shown that B_{6m} is a true resistance gene. It will be referred to as B_6. Knight's series of resistance genes B_1 to B_{10} referred to in the opening paragraph are all genes of major effect with intermediate resistance in the heterozygote. Minor genes play an important part in the full expression of the potential resistance of these genes. Recent work

completed in 1962 showed that the gene B_6 is additive in its effects when in combination with B_2, B_3, B_4 and B_5 but not with B_1 or B_7. B_6 with B_2 is the most effective combination.

2. MATERIALS AND METHODS

Two long staple, high quality cottons, Domains Sakel and Lambert of the species *G. barbadense* were used in this study. Both are fully susceptible to *X. malvacearum*. Certain bacterial blight resistance derivatives of these strains were also used. Lambert was derived by selection from Domains Sakel (Lambert, 1938) and therefore the name Sakel will be used for both in the presentation of the results.

All progenies grown in-season were sprayed with inoculum as described by Knight (1946) and graded. Out-of-season crops, grown to obtain two generations in one year, were also screened for resistance in a greenhouse using techniques described by Innes (1961).

Knight (1953) graded six families which had the following history: autotetraploid *G. arboreum* (produced by colchicine treatment of the diploid species) was crossed with Domains Sakel and subsequently backcrossed a further three times. This was followed by crossing to BLR 14/16 (a Sakel derivative of the genotype $B_2B_2b_6b_6$. Six single plants were taken from the last progeny and crossed with Domains Sakel ($b_2b_2b_6b_6$). The pedigree may be summarized as follows (tetraploid *arboreum* × Domains Sakel^4) × BLR 14/16) × Domains Sakel)F_1. The superscript 4 on Sakel denotes the number of crosses made to this variety. Knight's totals and genotypic classification are given below. The ratio obtained is in good agreement with a 1:1:2 two-gene backcross.

		Leaf disease grades								
	Grade of parent plants	3	4	5	6	7	8	9	10	12
Total of 6 families	'3'	4	22	2	7	28	—	—	—	70
Grouped totals			27		36					70
Expected: 1:1:2			33⅓		33⅓					66⅔
Genotype			$B_2b_2B_6b_6$		$B_2b_2b_6b_6$					$b_2b_2B_6b_6$ and $b_2b_2b_6b_6$

From this evidence it was clear that a strain homozygous for B_6 should be obtainable from grade '12' plants. The scheme set out below was therefore followed to achieve this result. It was not known at the time that plants homozygous for B_6 alone would be resistant.

The second backcross to $b_2b_2b_6b_6$ Sakel was not necessary to the present study but was introduced to improve the 'Sakel' qualities of the $b_2b_2B_6B_6$ stocks to be used ultimately in breeding commercial cottons. Selfed plants of the second backcross gave progenies which are referred to in this paper as F_2 families and selfed selections from within these F_2's are called F_3 progenies.

P $b_2b_2b_6b_6 \times B_2B_2B_6B_6$

$\downarrow$

F_1 $B_2b_2B_6b_6$

BC_1 $\times b_2b_2b_6b_6$

$\downarrow$

Resistant, discarded { 1 $B_2b_2B_6b_6$; 1 $B_2b_2b_6b_6$

Susceptible, backcrossed to $b_2b_2b_6b_6$ { 1 $b_2b_2B_6b_6$; 1 $b_2b_2b_6b_6$ } Test-crossed to $B_2B_2b_6b_6$ and screened in greenhouse. $b_2b_2b_6b_6$ plants discarded.

BC_2 Grown in field { 1 $b_2b_2B_6b_6$; 1 $b_2b_2b_6b_6$

All plants individually test-crossed to $B_2B_2b_6b_6$ and screened in the greenhouse. Plants identified as $b_2b_2B_6b_6$ were individually selfed giving F_2 progenies (Table 1).

$\downarrow$

F_2 Resistant 1 $b_2b_2B_6B_6$

Susceptible { 2 $b_2b_2B_6b_6$; 1 $b_2b_2b_6b_6$

F_3 Plants carrying B_6 were identified by greenhouse screening following crosses to $B_2B_2b_6b_6$. These plants were selfed and the F_3 type progenies are given in Table 2. Plants lacking B_6 were discarded.

3. RESULTS

Second backcross progenies were grown in the field out-of-season in 1958–59. Those families carrying B_6b_6 plants were identified by the greenhouse screening technique. F_2 seed was obtained from randomly chosen plants within these families. The selected plants were also test-crossed to a Sakel homozygous for B_2 in order to identify F_2 parents carrying B_6.

The F_2 families from second backcross plants were grown in the field in-season in 1959, sprayed and graded. Their corresponding test lines were examined in the greenhouse. The F_2 families in the field were expected to be uniformly fully susceptible because none carried the gene B_2. However, it was found that progenies derived from selfed B_6b_6 plants were segregating into 'resisters' and 'susceptibles' (Table 1*a*) in a proportion of one to three respectively. Progenies derived from b_6b_6 plants were entirely graded '12'. Table 1*b* gives a second set of families showing the same type of segregation from single plants similarly derived but which were not identified as to genotype in test crosses. However, it is most likely that B_6 was present in these families also.

The individual families were small in size and the ratio of 'resisters' to 'susceptibles' varied considerably. Nevertheless the group totals in both sets of families strongly suggested the segregation of a single recessive gene. These results indicated that the resistant plants were B_6B_6 but conclusive tests were needed. Plants were therefore chosen at random from the F_2 families and selfed to give seed for F_3 progenies. The same selections were also backcrossed to $b_2b_2b_6b_6$ Sakel and to the $B_2B_2b_6b_6$ Sakel test line. The F_3 families and the equivalent backcrosses were

https://doi.org/10.1017/S0016672300003773 Published online by Cambridge University Press

sown in the field, sprayed and graded. The parental plant genotypes were checked in the test lines raised in the greenhouse. All backcrosses to $b_2b_2b_6b_6$ Sakel gave fully susceptible families. All selfed plants, identified in the test crosses as being homozygous for B_6, gave progenies in the grades '7–9'. Selfed B_6b_6 plants once

Table 1. *Leaf disease grades of 'F_2' families from plants of the second Sakel backcross to* ($B_2B_2B_6B_6 \times b_2b_2b_6b_6$)

(*a*) Progenies derived from plants known to be genotypically $b_2b_2B_6b_6$:

Family no.	Parent grade	Leaf disease grade						Grouped totals	
		6	7	8	9	10	12	6–9	12
BA 630/59	12	—	—	2	—	—	9	2	9
634/59	12	—	—	1	—	—	2	1	2
636/59	12	—	—	—	—	—	10	—	10
710/59	12	1	1	—	—	—	7	2	7
718/59	12	1	1	1	—	—	7	3	7
731/59	12	—	3	—	—	—	7	3	7
750/59	12	—	—	—	—	—	10	—	10
753/59	12	—	1	—	—	—	8	1	8
771/59	12	—	4	2	—	—	4	6	4
777/59	12	1	1	—	—	—	8	2	8
Total		3	11	6	—	—	72	20	72
							Expected (1:3)	23	69

(*b*) Progenies derived from plants probably genotypically $b_2b_2B_6b_6$:

BA 611/59	12	—	1	2	—	—	7	3	7
614/59	12	—	—	1	—	—	8	1	8
638/59	12	—	1	—	—	—	9	1	9
650/59	12	—	3	—	—	—	5	3	5
654/59	12	—	3	—	—	—	6	3	6
658/59	12	1	2	—	—	—	3	3	3
659/59	12	—	1	1	1	—	7	3	7
751/59	12	—	2	1	—	—	7	3	7
756/59	12	—	4	1	—	—	5	5	5
758/59	12	—	2	1	—	—	7	3	7
775/59	12	1	—	1	—	—	7	2	7
778/59	12	—	—	1	—	—	9	1	9
Total		2	19	9	1	—	80	31	80
							Expected (1:3)	$27\frac{3}{4}$	$83\frac{1}{4}$

again segregated into one 'resister' to three 'susceptibles' (see Table 2), thus confirming the findings from the F_2 families.

Although it is evident from the data in table 2b that a single major gene is segregating, there is a clear distortion from the expectation on a 1:3 basis. The deviation is significant, the families being homogeneous; the reason for the distortion is unknown.

https://doi.org/10.1017/S0016672300003773 Published online by Cambridge University Press

Table 2. *Leaf disease grades of 'F_3' families from selfed plants of the 'F_2's of Table* 1

(*a*) Progenies of self-bred B_6B_6 plants:

Family no.	Leaf disease grade 7	8	9	10	12	Grouped totals 7–9	10–12
BA 511/60	61	—	—	—	—	62	—
527/60	20	21	—	—	—	41	—
577/60	49	9	—	—	—	58	—
612/60	—	13	8	—	—	21	—
Totals	131	43	8	—	—	182	—

(*b*) Progenies of self-bred B_6b_6 plants:

BA 512/60	7	5	—	2	44	12	46
513/60	2	8	—	—	35	10	35
514/60	3	5	—	—	37	8	37
515/60	4	8	1	—	44	13	44
536/60	1	10	—	1	48	11	49
574/60	8	3	—	—	47	11	47
575/60	9	1	—	—	41	10	41
610/60	—	11	2	—	41	13	41
611/60	—	9	4	—	39	13	39
613/60	—	12	2	—	35	14	35
614/60	—	4	3	—	35	7	35
Totals	34	76	12	3	446	122	449
					Expected (1:3)	$142\frac{3}{4}$	$428\frac{1}{4}$

(*c*) χ^2 test:

	χ^2	D.F.	*P*
Deviation from 1:3 ratio	4·02	1	0·05–0·02
Heterogeneity	3·41	10	0·95

A large number of families were grown for testing the behaviour of B_6. None were found which contradicted the observation that B_6 is a recessive resistance gene. Table 3 is given to show the grouped totals of two sets of data derived from selfed B_6b_6 plants confirming earlier results. It is interesting to note that a similar shortage of 'resisters' occurred in the 1962 data.

Table 3. *Grouped families derived from selfed B_6b_6 plants*

	Leaf disease grade 6	7	8	9	10	12	Expected 1 : 3	
Bulk of 5 families	—	38	23	4	—	207		
			65			207	68	204
Bulk of 19 families	60	247	43	2	19	1261		
			351			1281	408	1224

https://doi.org/10.1017/S0016672300003773 Published online by Cambridge University Press

The final assessment of B_6 was made by observing its behaviour when segregating with B_2. The cross $b_2b_2B_6B_6 \times B_2B_2b_6b_6$ was made and F_1 plants were selfed. Four F_2 families were raised and the results on grading are given in Table 4.

Table 4. *F_2 progenies derived from a cross between $B_2B_2b_6b_6$ Sakel and $b_2b_2B_6B_6$ Sakel*

(*a*) Classification of families:

Family no.	Leaf disease grade 3	4	5	6	7	8	9	10	12	Total plants
BA 5/62	—	11	4	1	4	3	—	—	5	28
6/62	1	16	7	2	6	2	—	—	6	40
7/62	—	19	18	—	9	6	4	—	13	69
8/62	—	11	2	—	5	—	3	—	6	27
Total	1	57	31	3	24	11	7	—	30	164

(*b*) Families divided at points of minimum frequency and grouped:

	Observed Leaf grade groups 3–6	6–9	12	Expected ratio 9 : 4 : 3
BA 5/62	15·5	7·5	5·0	15·75 : 7·00 : 5·25
6/62	25·0	9·0	6·0	22·50 : 10·00 : 7·50
7/62	37·0	19·0	13·0	38·80 : 17·20 : 13·00
8/62	13·0	8·0	6·0	15·20 : 6·80 : 5·00
Total	90·5	43·5	30·0	92·25 : 41·00 : 30·75

(*c*) χ^2 test:

	χ^2	D.F.	*P*
Deviation from 9:4:3 ratio	0·20	2	0·9
Heterogeneity	1·54	3	0·6

The families presented in Table 4 are clear evidence of the segregation of one dominant gene, B_2 (B_2b_2 is slightly less resistant than the homozygote B_2B_2) and one recessive, B_6 where an observed total of 134 'resisters' to 30 'susceptibles' is in close agreement with an expectation of $133\frac{1}{4}$ to $30\frac{3}{4}$ in the same classes respectively for a two gene segregation of 13:3. The resistant component can be further subdivided at a minimum frequency at grade '6'. Excellent agreement is thus obtained with a 9:4:3 ratio, where the genotypes in these classes are as follows:

$1\ B_2B_2B_6B_6$
$2\ B_2b_2B_6B_6$
$2\ B_2B_2B_6b_6$
$4\ B_2b_2B_6b_6$ } 9

Group 1: B_2–B_6 gives high resistance

$1\ B_2B_2b_6b_6$
$2\ B_2b_2b_6b_6$
$1\ b_2b_2B_6B_6$ } 4

Group 2: B_2 or B_6B_6 give medium resistance

$2\ b_2b_2B_6b_6$
$1\ b_2b_2b_6b_6$ } 3

Group 3: Absence of B genes or B_6b_6 give full susceptibility

https://doi.org/10.1017/S0016672300003773 Published online by Cambridge University Press

4. DISCUSSION

Nothing conclusive is known concerning the mechanism of resistance to bacterial blight. Under the favourable climatic conditions of the northern Sudan, Knight was able to distinguish the individual action of each of the B genes he described and accordingly referred to them as major genes. However, in other parts of Africa, e.g. Uganda and Tanganyika, other workers have been unable to follow the segregation of the same genes. This has been attributed to a less uniform incidence of the disease under their conditions. The distinction between major and minor genes in this instance depends upon environment (Hutchinson, 1959). Certain B gene combinations do give additive effects under Sudan conditions. When Knight studied B_2 and B_3 he found that in combination they gave greater resistance than when alone. This is no longer true of varieties he developed carrying B_2 and B_3 since maintained by selfing or of similar varieties developed in subsequent work. This has been discussed by Gunn (1961). However, a study by Innes (1963) of two stocks, homozygous for B_2B_3, but differing greatly in their resistance, showed that this difference was entirely attributable to a favourable minor gene background in the one stock which was lacking in the other. B_6, reported here, does not differ in kind from the other B genes but has a much more marked interaction in combination.

SUMMARY

A study of the gene B_{6m}, previously described as a modifier, revealed that it is a recessive resistance gene of moderate effect when homozygous. Its value in enhancing resistance to bacterial blight when in combination with other genes, in particular B_2, is emphasized. The symbol for the gene is simplified to B_6.

Grateful acknowledgement is made to the Chief of Agricultural Research Division, Republic of the Sudan, for permission to publish this paper.

REFERENCES

GUNN, R. E. (1961). Bacterial blight of cotton. Loss of disease resistance in certain gene combination in Sakel cotton. *Emp. Cott. Gr. Rev.* **38,** 284.

HUTCHINSON, J. B. (1959). *The Application of Genetics to Cotton Improvement*, 53. Cambridge Univ. Press.

INNES, N. L. (1961). Breeding bacterial blight resistant cotton in the Sudan by combining green-house inoculation with field spraying. *Emp. Cott. Gr. Rev.* **38,** 92

INNES, N. L. (1963). Bacterial blight of cotton. Resistance conferred by the gene combination B_2B_3 in the Sudan. *Emp. Cott. Gr. Rev.* **40,** 117.

KNIGHT, R. L. (1946). Breeding cotton resistant to blackarm disease. Part 2. Breeding methods. *Emp. J. exp. Agric.* **14,** 161.

KNIGHT, R. L. (1953). The genetics of blackarm resistance. 9. The gene B_{6m} from *Gossypium arboreum*. *J. Genet.* **51,** 270.

KNIGHT, R. L. (1957). Blackarm disease of cotton and its control. *Plant Protection Conference*, 1956, p. 53. London: Butterworth.

LAMBERT, A. R. (1938). New Sakel strains in the Anglo-Egyptian Sudan. *Emp. Cott. Gr. Rev.* **15,** 14.