GENETIC RESISTANCE TO THE CEREAL CYST NEMATODE (*HETERODERA AVENAE*)

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1. INTRODUCTION

THE cereal cyst nematode (*Heterodera avenae* Woll.) is a soil-borne parasite of barley, oat and wheat, and is characterised by the development of the adult females into lemon-shaped cysts which are clearly visible on the roots of the host plant. Each cyst contains several hundred larvae and provides the source of infection for the following year.

In certain genotypes of barley, the female fails to reach maturity and consequently no cysts are found on the roots; this resistance is being introduced into modern barley cultivars. Up to the present, breeders have used two main sources of resistance. The existence of nematode races capable of attacking both sources, and the availability of other types of resistance, necessitate a knowledge of the genetic inter-relationships of various factors for resistance to different pathotypes before the next stage in the resistance breeding programme can be planned. Populations of the cereal cyst nematode vary in pathogenicity, *i.e.* in their ability to form cysts on the same host (Andersen, 1961; Cotten, 1963, 1967; Kort *et al.*, 1964). The two races, designated 1 and 2 in Denmark and Britain, and A and C in the Netherlands, make up the bulk of the British populations. In this investigation the inheritance of resistance of six unrelated barley genotypes was studied using two populations of the cereal cyst nematode known to differ in their pathogenicity.

2. MATERIALS AND METHODS

A description of the resistant parents employed, the susceptible control Cb 545 (Rika) and their reaction to races 1 and 2 are presented in table 1.

The parents, F_1 hybrids, and F_2 segregates were tested for nematode reaction against two nematode populations designated 1 and 2 which were similar in pathogenicity reaction to races 1 and 2. Individual seedlings were planted in $3\frac{1}{2}$ in. diameter (8 cm.) pots of infested soil in March. The pots were arranged in a randomised block layout, with the populations treated as two separate experiments. At ear emergence (10-12 weeks after planting), the number of white cysts on the root system of each plant was determined by the method described by Cotten (1967).

The classification of F_2 segregates was based on the reaction of the homozygous resistant and susceptible parents and heterozygous F_1 plants grown in a randomised block layout, using the same source of soil inoculum for all blocks in each experiment. After a square root transformation, the mean and standard deviation were computed for each parent and F_1 hybrid population, and members of the F_2 populations were classified according to their highest level of probability of being included in a particular parental group. In no case did an F_2 segregate fall outside the 95 per cent. level of probability of belonging to a particular parental class.

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TABLE 1

Designation and origin of seven barley genotypes and their reaction to races 1 and 2

W.P.B.S. accession number	Varietal name or other designation	Source of seed	Cereal cyst nematode reaction	
			Race 1	Race 2
Cb 545	Rika	W.P.B.S. stock	+	+
Cb 917	Fero	Professor Sigurd Andersen, Copenhagen, Denmark	−	+
Cb 918	Drost		−	+
Cb 824	No. 191		−	−
Cb 1018	No. 14		−	−
Cb 1022	C.I. 8334	Dr Van Essen Wageningen, Netherlands	−	−
Cb 1023	C.I. 3902		−	−

+ = Cysts present. − = Cysts absent.

TABLE 2

The range of number of cysts produced on parents, F_1, and F_2 plants of a 5 parent diallel when tested against two populations of the cereal cyst nematode

Cross		Range of the number of cysts produced per plant			
P_1	P_2	P_1	P_2	F_1	F_2
Population 1					
Cb 545 × Cb 917		220-767	1-32	30-46	1-484
Cb 545 × Cb 918		220-767	2-38	15-53	0-941
Cb 545 × Cb 824		220-767	0	0-1	0-499
Cb 545 × Cb 1018		220-767	0	2-12	0-672
Cb 917 × Cb 918		1-32	3-28	5-24	0-27
Cb 917 × Cb 824		1-32	0	0	0-31
Cb 917 × Cb 1018		1-32	0	0-1	0-440
Cb 918 × Cb 824		2-38	0	0-1	0-2
Cb 918 × Cb 1018		2-38	0	0-4	0-606
Cb 824 × Cb 1018		0	0	0-1	0-4
Population 2					
Cb 545 × Cb 917		36-186	30-225	26-145	24-206
Cb 545 × Cb 918		36-186	15-222	19-150	15-216
Cb 545 × Cb 824		36-186	0	0	0-85
Cb 545 × Cb 1018		36-186	0-2	0-9	0-104
Cb 917 × Cb 918		30-225	15-222	25-100	20-307
Cb 917 × Cb 824		30-225	0	0	0-232
Cb 917 × Cb 1018		30-225	0-2	0-3	0-235
Cb 918 × Cb 824		15-222	0	0	0-199
Cb 918 × Cb 1018		15-222	0-2	0-2	0-333
Cb 824 × Cb 1018		0	0-2	0	0-9

3. RESULTS

(i) *Interrelationships of genes for resistance*

The range of cyst numbers produced on the parents, F_1, and F_2 segregates when tested against population 1 and 2, are given in table 2. Analysis of variance followed by Duncan's Multiple Range Test applied to all the parent and F_1 data (but excluding Cb 824 and Cb 1018 and their hybrids)

showed that, when tested against population 1, Cb 917 and Cb 918 and their hybrids, although not fully resistant like Cb 824 and Cb 1018, had significantly fewer cysts than Cb 545. Tested against population 2, Cb 545, Cb 917 and Cb 918 produced a similar number of cysts, and all three could be considered as fully susceptible. The number of cysts found on Cb 545, when tested against population 1, ranged from 220 to 767 and, against population 2, from 36 to 186, the actual number of cysts produced depending partly on the level of inoculum in the soil.

TABLE 3

Observed and expected F_2 frequencies with levels of probabilities of data fitting hypothesis outlined in text

Population 1

Parents	Cb 917	Cb 918	Cb 824	Cb 1018	F_1 hybrid Cb 918 × Cb 824
	R S	R S	R S	R S	R S
Cb 545	O = 12 : 8 $P_{3:1} > 0{\cdot}10$	O = 33 : 7 $P_{3:1} > 0{\cdot}20$	O = 21 : 4 $P_{3:1} > 0{\cdot}20$	O = 12 : 8 $P_{3:1} > 0{\cdot}10$	O = 12 : 3 $P_{3:1} > 0{\cdot}50$
Cb 917	— —	O = 20 : 0 No test	O = 20 : 0 No test	O = 17 : 3 $P_{15:1} > 0{\cdot}10$	
Cb 918	— —	— —	O = 44 : 0 No test	O = 18 : 2 $P_{15:1} > 0{\cdot}30$	
Cb 824	—	—	—	O = 20 : 0 No test	

Population 2

Parents	Cb 917	Cb 918	Cb 824	Cb 1018
	R S	R S	R S	R S
Cb 545	O = 0 : 19	O = 0 : 39	O = 18 : 7 $P_{3:1} > 0{\cdot}70$	O = 14 : 6 $P_{3:1} > 0{\cdot}50$
Cb 917	—	O = 0 : 20	O = 8 : 10 $P_{3:1} < 0{\cdot}01$	O = 14 : 6 $P_{3:1} > 0{\cdot}50$
Cb 918	—	—	O = 32 : 11 $P_{3:1} > 0{\cdot}90$	O = 13 : 7 $P_{3:1} > 0{\cdot}30$
Cb 824	—	—	—	O = 20 : 0 No test

When tested against population 1 the resistance of Cb 824 was epistatic to the partial resistance of Cb 917 and Cb 918, and dominant to the susceptibility of Cb 545. The partial resistance of Cb 917 and Cb 918 to population 1 was dominant to the fully susceptible reaction of Cb 545. Cb 824 and Cb 1018 gave similar results against both populations 1 and 2, although the resistance of Cb 1018 was never as complete as that of Cb 824, and similarly the F_1 hybrids involving Cb 1018 developed more cysts than those involving Cb 824.

The F_2 segregants were classified into resistant and susceptible types according to the reaction of the more resistant parent and F_1 hybrids in each cross against each population. The maximum number of cysts produced on either the homozygous resistant parent or F_1 hybrids was the approximate demarcation line between resistance and susceptibility. Thus the resistant group contained both homozygous resistant and heterozygous plants. The observed frequencies of resistant and susceptible plants classified on this basis are given in table 3; in addition, the expected ratios and results of χ^2 tests

are given, assuming that resistance to a particular pathotype is controlled by a single gene and is dominant to partial resistance and susceptibility, and that the partial resistance of Cb 917 and Cb 918 to population 1 is also controlled by a single dominant gene, independent of the gene for resistance in Cb 824 and Cb 1018. It is evident from the segregation of the F_2 progenies of Cb 917 × Cb 1018, and Cb 918 × Cb 1018, when tested against population 1, that the gene for resistance in Cb 1018 is at a different locus from that of Cb 917 and Cb 918; there is no evidence to suggest that the resistance genes of Cb 917 and Cb 918 are at different loci, although the size of the F_2 populations tested is too small to preclude the possibility of close linkage.

Although no susceptible plants were recovered from the cross of Cb 917 and Cb 918 with Cb 824 when tested against population 1, the results of crossing the F_1 hybrid (Cb 918 × Cb 824) to the susceptible Cb 545 indicate that the genes for resistance in Cb 918 and Cb 824 are not at the same locus.

TABLE 4

Segregation in the 9th-11th back-cross generations of the cross Cb 824 × susceptible recurrent parent when tested against race 1 and 2 alternately

Back-cross (generation)	Test population	Observed ratio		Expected ratio after back-crossing resistant progeny to susceptible recurrent parent: Single locus		Independent loci		χ^2 (1 : 1 ratio) probability
		R	S	R	S	R	S	
Bc_9	2	30	30	1	1	1	1	0·99
Bc_{10}	1	24	24	1	1	1	3	0·99
Bc_{11}	2	13	12	1	1	1	3	0·80-0·90

When the F_2 populations were tested against population 2, the results indicated that a single dominant gene for resistance is operating in Cb 824 and Cb 1018, although the results from the cross Cb 917 × Cb 824 do not conform with this hypothesis ($P<0{\cdot}01$). There is no evidence in these data of any segregation in the crosses Cb 824 × Cb 1018 (table 2).

Data derived from a breeding programme designed to develop a cereal cyst nematode resistant variety show that the resistance of Cb 824 to populations 1 and 2 is due to the same gene. F_1 hybrids from Cb 824 crossed with a susceptible parent were back-crossed for eleven generations to a recurrent parent susceptible to both populations. Resistant back-cross hybrids were retained for further back-crossing on the basis of their reaction to mixed eelworm populations. However, back-cross hybrids were tested for the last three generations against population 1 and 2 in alternate generations (table 4).

The genetic relationship of the resistance of Cb 824 and Cb 1018 was clarified in a further series of crosses in which two additional resistant parents were included (table 5). In this experiment the parents, F_1 and F_2 progenies were tested against population 2. In the F_2 progeny of the cross Cb 824 × Cb 1018, two plants were recovered which could be classed as susceptible, indicating that the resistance genes were located at different loci. In the F_2 progenies of the other five crosses the cyst production on all the plants fell either within, or lay very close to, the range of reaction of the parents and F_1 hybrids.

TABLE 5

Cyst production on parents, F_1 hybrids, and F_2 progeny from intercrosses of four cereal cyst nematode resistant genotypes. Tested against population 2. (Cb 545 Rika = susceptible control with 3-82 cysts/plant)

Cross		Range of the number of cysts produced/plant				F_2 segregation	
						Observed ratio	
P_1	P_2	P_1	P_2	F_1	F_2	R : S	$P\chi^2_{15:1}$
Cb 824	Cb 1018	0	0-2	0-2	0-22	58 : 2	> 0·30
Cb 824	Cb 1022	0	0-1	0	0-1	60 : 0	—
Cb 824	Cb 1023	0	0-1	0	0-1	60 : 0	—
Cb 1018	Cb 1022	0-2	0-1	0	0-4	59 : 0	—
Cb 1018	Cb 1023	0-2	0-1	0	0-2	60 : 0	—
Cb 1022	Cb 1023	0-1	0-1	0	0-1	60 : 0	—

(ii) *Linkage relationships of the resistance factor in Cb* 824

The locus of the major gene for resistance in Cb 824 which is effective against pathotypes 1 and 2 has been designated *Ha*. Hybrids were obtained between the two-row genotype Cb 825, having resistance from Cb 824, and seven genotypes which are susceptible to the cereal cyst nematode and homozygous for reciprocal translocations involving each of the seven chromosomes. The seven translocation stock parents and the techniques involved were the same as those used by Hayes and Rana (1966).

In the segregation of the F_2 progenies there was no positive linkage of *Ha* with any of the break points in the seven translocation stocks. However, five of the susceptible translocation stocks used as parents were six-row (*ha ha vv*). In the F_2 progeny of the five crosses, which included these six-row parents, an excess of six-row susceptible and two-row resistant phenotypes was recovered (table 6).

TABLE 6

The phenotypes of F_2 progenies from five families segregating for head type (Vv), *and reaction to the cereal cyst nematode* (Ha ha)

Cereal cyst-nematode reaction	Resistant (*Ha*)		Susceptible (*ha*)	
Head type	Two-row (*V*)	Six-row (*v*)	Two-row (*V*)	Six row (*v*)
Total for five families	237	22	21	68

Partitioning of the chi square values (table 7*a*) clearly indicated that linkage between the two loci was responsible for the disturbed F_2 ratio. The heterogeneity test (table 7*b*) indicated that the data from the five families are homogeneous for each of the three components.

The recombination value between *Ha* and *V* was estimated to be $13{\cdot}2 \pm 1{\cdot}96$. Since the *Vv* locus is known to be located on chromosome 2, these results demonstrate that the gene (*Ha*) responsible for nematode resistance of Cb 824 (No. 191) is also located on chromosome 2. The position of the *Ha ha* locus has been confirmed using another chromosome 2 genetic marker, the character " liguleless " (*li*). The F_2 progeny of the cross between Cb 1113, a six-row susceptible parent without ligules (*v, ha, li*) and Cb 825, a two-row resistant parent with ligules (*V, Ha, Li*), in addition to confirming the linkage between *Vv* and *Ha ha*, also gave evidence of linkage between *Li li* and *Ha ha* (table 8).

TABLE 7 (*a*)

Chi square values for segregation of cereal cyst nematode reaction (Ha ha), *head type* (Vv), *and linkage*

Character	d.f.	χ^2 value	Probability
Segregation of *Ha ha*	1	0·14	> 0·70
Segregation *Vv*	1	0·25	> 0·50
Linkage	1	165·59	< 0·001
Total	3	165·98	< 0·001

TABLE 7 (*b*)

Heterogeneity chi square test of data from five families

	χ^2 values			
	Ha	*V*	Linkage	d.f.
Total	3·04	1·41	172·56	5
Deviation	0·14	0·25	165·59	1
Heterogeneity	2·90	1·16	6·97	4
Probability	> 0·50	> 0·80	> 0·10	–

TABLE 8

The phenotypes of an F_2 progeny segregating for the character liguleless, and reaction to cereal cyst nematode

Resistant (*Ha*)		Susceptible (*ha*)	
Ligules (*Li*)	Liguleless (*li*)	Ligules (*Li*)	Liguleless (*li*)
112	17	19	17

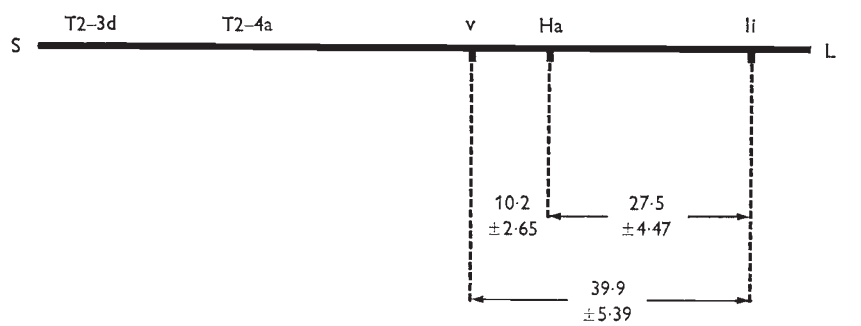
TABLE 9

Chi square values for segregation of the character liguleless (Li li), *cereal cyst nematode reaction* (*Ha ha*) *and linkage*

	d.f.	χ^2 value	Probability
Segregation of *Ha ha*	1	0·89	> 0·30
Segregation of *Li li*	1	1·70	> 0·10
Linkage	1	16·60	< 0·001
Total	3	19·19	< 0·001

Partitioning of the chi square values (table 9) again indicated that linkage between the two loci was responsible for the disturbed F_2 ratio.

Plotting the three point linkage values indicates that the *Ha* locus lies between *li* and *v* on chromosome 2.



4. Discussion

Some difficulty was experienced in classifying certain plants as either resistant or susceptible, partly due to environmental variation and partly due to genetic variation in aggressiveness in the nematode. A wide range in the number of cysts produced on genetically homozygous susceptible genotypes was encountered even where plants were grown under uniform conditions, making it impossible to arrive at an overall numerical line of demarcation for resistance and susceptibility. Because of this, a statistical test was applied and by this method, members of the F_2 populations were classified according to their highest level of probability of being included in a particular parental group. The lengthy procedure for determining the nematode reaction of plants precluded the screening of large F_2 populations and, therefore, critical data are of necessity based on relatively low numbers of plants.

The results clearly show that there is a system of major genes for resistance to the cereal cyst nematode operating in the host. In the six genotypes used, resistance is controlled by at least three and possibly more genes at different loci. There is a single dominant gene *Ha* for resistance to races 1 and 2 in Cb 824 (No. 191) which is located on chromosome 2, 27 units from *li* (liguleless) and 10 units from *v* (six-row). There is also a dominant gene for resistance to race 1 only in Cb 917 (Fero) and Cb 918 (Drost), and the data show that this gene in Cb 918 is independent of, or only loosely linked to, *Ha*. This result is confirmed by the work of Andersen and Andersen (1968). The monogenic resistance of Cb 1018 (No. 14) to races 1 and 2 is also clearly at a different locus to the Cb 917 and Cb 918 genes. All the available evidence indicates that the genes for resistance in Cb 917 and Cb 918 are at the same locus.

In the second series of tests which included the parents Cb 824 and Cb 1018, two susceptible plants were recovered in the F_2 population of the cross Cb 824 × Cb 1018 (table 5). Statistical analysis showed that 95 per cent. of the F_2 population of that cross could be expected to fall within the range 0-3 cysts. The two plants in question bearing 19 and 22 cysts respectively lay far outside this range, but were within the 95 per cent. probability range for the susceptible check variety Cb 545 (0 to 71 cysts), thus providing strong evidence that the resistance genes of Cb 824 and Cb 1018 are not at the same locus.

The gene for resistance in Cb 824 is not the same as those in Cb 1022 (C.I. 8334) and Cb 1023 (C.I. 3902), since unlike Cb 1022 and Cb 1023, the Cb 824 gene is not effective against the Dutch race B (Kort *et al.*, 1964). However, the resistance of Cb 1022 and Cb 1023 is no more effective than that of Cb 824 against British races of cereal cyst nematode, since our tests have recently shown that all three genotypes are susceptible to the British race 3. The relationship of the genes for resistance in Cb 1022 and Cb 1023 with each other and with either Cb 824 or Cb 1018 is not fully established. With the data available it is evident that genes at the same locus or closely linked loci are involved.

The evidence for the location of *Ha* on chromosome 2 is clear. The genes for resistance in Cb 1018, Cb 1022 and Cb 1023 are also located on this chromosome, since our results indicate that these genes are also closely linked to *Ha*. If this is the case, the close location of loci controlling resistance to cereal cyst nematode on chromosome 2 would reflect the genetic situation

with regard to mildew resistance where many factors for resistance to various physiologic races of the pathogen are found closely linked on chromosome 5 in barley (Moseman, 1963).

The gene *Ha* has now been introduced into adapted genotypes of spring barley which are effective against most of the British populations of cereal cyst nematode. However, additional genes for resistance will need to be found should race 3 or any as yet undetected race become prevalent as a result of growing such varieties on a large scale.

5. Summary

1. The genetic basis of resistance of six barley genotypes to two populations of the cereal cyst nematode which differed in their pathogenicity has been investigated; nematode resistance in these genotypes is characterised by the inability of the female to reach maturity in the root tissue.

2. Genetic analysis of F_2 populations showed that in each of four genotypes, resistance to a particular nematode population was controlled by a single dominant gene and in one of them (Cb 824 = No. 191) resistance to both populations was controlled by the same gene.

3. Resistance was found to be controlled by genes at a minimum of three loci, with close linkage of two of these loci.

4. The dominant gene for resistance in Cb 824, designated *Ha*, was found to be located on chromosome 2, and has a recombination value of $10{\cdot}2 \pm 2{\cdot}65$ with the *v* (6-row) locus and $27{\cdot}5 \pm 4{\cdot}47$ with the *li* (liguleless) locus.

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6. References

ANDERSEN, S. 1961. Resistens mod havreal *Heterodera avenae*. Meddr K. Vet.-og Landbohøjsk. No. 68.

ANDERSEN, K., AND ANDERSEN, S. 1968. Inheritance of resistance to *Heterodera avenae* in barley. *Nematologica, 14*, 128-130.

COTTEN, J. 1963. Resistance in barley and oats to the cereal root eelworm *Heterodera avenae Wollenweber*. *Nematologica, 9*, 81-89.

COTTEN, J. 1967. Cereal root eelworm pathotypes in England and Wales. *Pl. Path., 16*, 54-59.

HAYES, J. D., AND RANA, M. S. R. 1966. Genetic resistance to DDT in *Hordeum*. 1. Linkage studies in diploid barley. *Heredity, Lond., 21*, 581-593.

KORT, J., DANTUMA, G., AND VAN ESSEN, A. 1964. On biotypes of the cereal root eelworm (*Heterodera avenae*) and resistance in oats and barley. *Neth. J. Pl. Path., 70*, 9-17.

MOSEMAN, J. G. 1963. Function of the host and pathogen genetic systems in elucidating disease development. Barley Genetics 1. (Proc. First Internal. Barley Genetics Symp. Wageningen 1963), 215-221.