Theor. Appl. Genet. 61, 209–214 (1982)

Evolution of Resistance to *Rhynchosporium secalis* (Oud.) Davis in Barley Composite Cross II

O. Muona and R. W. Allard
Department of Genetics, University of California, Davis, Calif. (USA)

R. K. Webster
Department of Plant Pathology, University of California, Davis, Calif. (USA)

Summary. Changes in resistance to scald disease which occurred in barley composite Cross II over 45 generations were analyzed genetically. This population, which was synthesized in 1929 by pooling equal numbers of F_1 seeds from 378 pair wise crosses among 28 barley varieties, has subsequently been grown at Davis, California under standard agricultural conditions without conscious selection. Progenies derived from self-pollinated seeds from random plants taken from four generations (F_8, F_{13}, F_{23}, and F_{45}) were tested against four different races of scald (40, 61, 72, and 74), and rated as resistant, susceptible or segregating. Striking increases in the frequency of families resistant to races 40, 61, and 74 occurred in CC II. A test for randomness showed that quadruply susceptible and triply resistant families were more common than expected under the assumption that resistance to different races is independent. Positive correlations were found between resistance to races 40, 61, and 74, but resistance to race 72 was independent of resistance to all other races. Possible reasons for these correlations are discussed.

Key words: Inheritance of resistance – Plant breeding

Introduction

In a study of barley scald disease, caused by *Rhynchosporium secalis* (Oud.) Davis, Jackson et al. (1978) found that the frequency of resistant individuals increased significantly in later generations of Composite Cross II (CC II), whereas little change occurred in the frequency of resistant plants in CC V and CC XXI. The increases in frequency of resistant plants in CC II were to three of the four races studied. Studies of the pathogen have shown that its racial composition is complex. At least 75 different physiological races occur in the Sacramento and San Joaquin Valleys of California (Jackson and Webster 1976a) and it is known that large changes take place in the racial composition of the pathogen over time (Jackson 1979). It has also been shown that the racial composition of populations of scald can change rapidly in controlled greenhouse experiments (Jackson and Webster 1976b). The four races used in the study by Jackson et al. (1978), and also in this study, therefore represent only a part of the pathogen population in nature. These four races are, however, diagnostic and they identify the major types of virulence that are known in the pathogen.

There is substantial experimental evidence that correlations can develop between different genetic loci in highly self-pollinating populations such as CC II (Allard et al. 1968; Clegg et al. 1972; Weir et al. 1972; Weir et al. 1974). The purpose of the present study was to determine whether the observed changes in resistance to different races are correlated. The results are discussed with respect to known genes for resistance to scald.

Materials and Methods

Composite Cross II was synthesized in 1929 by mixing equal numbers of F_1 seeds from 378 hybrids obtained by making pair wise crosses among 28 varieties of barley (Harlan and Martini 1929). The population has subsequently been grown annually at Davis, California in large plots under standard agricultural conditions without conscious selection. Families for the present study were obtained by space planting random samples of seeds from generations F_8, F_{13}, F_{23}, and F_{45} and harvesting seeds from single plants. One hundred thirty-two families from each generation were scored for resistance in greenhouse tests. Methods of inoculation and scoring have been described by Jackson and Webster (1976a). Four lots of 10 seeds each were planted per family in metal flats. Two weeks after planting the flats were inoculated with either race 40, 61, 72, or 74 (ATCC34256, ATCC34277, ATCC34288, and ATCC34290, respectively). The inoculum concentration was 2×10^5 spores/ml. Disease ratings were made after two weeks of incubation. Seedlings were classified in five categories on the basis of level of infection. Ratings 0, 1, and

0040-5752/82/0061/0209/$ 1.20

2 were considered to indicate resistance and ratings 3 or 4 to indicate susceptibility. Families which contained only individuals with scores of 0, 1, or 2 were classified as resistant, those in which only types 3 or 4 individuals were observed were designated as susceptible (S) and those in which both types 0–2 and 3–4 were found were classified as segregating (SEG). It should be noted that the proportion of segregating families will be underestimated due to the small family size. There are three possible family types for each of the four races and thus $3^4 = 81$ possible family types in total.

Results

The basic data of the experiment are given in Tables 1 and 2. Table 1 lists the family types observed in four generations of CC II; it also gives the family types of the parents reported by Jackson et al. (1978). Table 2 gives the frequencies of resistant, segregating, and susceptible families observed in the present study and the frequencies of resistant and susceptible individuals reported by Jackson et al. (1978) in four generations (F_7, F_{15}, F_{25}, F_{47}) of CC II. Because resistance is usually dominant to susceptibility (see Habgood and Hayes 1971) most segregating families are expected to have arisen from resistant plants. The two data sets can therefore be made approximately comparable by adding the frequencies of resistant and segregating families of the present study and comparing the sum with the frequency of resistant individuals in the earlier study. Alternatively the frequencies of susceptible families in the present study can be compared directly with the frequency of susceptible individuals in the study of Jackson et al. (1978). The data in Table 2 show that the main difference between the two studies is that resistant plants were less frequent in generation F_{45} of the present study than in generation F_{47} of the earlier study. Scoring for disease resistance, which is subjective, was by different observers in the two studies and it is therefore possible that the difference between generations F_{45} and F_{47} reflect systematic differences among observers in scoring disease levels. It is also possible that inoculation and incubation conditions differed in the two studies. Neither of these explanations seems likely, however, in view of the very similar results observed in the earlier generations of the two studies. Thus the higher frequency of resistant plants in generation 47 may possibly represent a real increase over the frequency observed in generation 45. This possibility is consistent with the striking increase in the frequency of resistant plants which occurred in the later generations of CC II.

It can be seen from Table 1 that 30 among the 81 possible family types were observed. Only six different family types occurred in the parents, and also in generation 13. However, in generations F_8, F_{23}, and F_{45} of CC II, the number of different family types was much larger (16, 17, and 17, respectively).

Family type SSSS (susceptible to all four races) was the most common type observed (20 out of 28, or 71%) in the parents of CC II. This family type was also the most common in generations F_8, F_{13}, and F_{23} (74, 89, and 59%, respectively). However, by generation F_{45} it had become infrequent (16 out of 132 families or 12%).

Table 1. Observed numbers of family types in the parents and four generations of CC II

Race				Number of parent varieties[b]	Generation			
40	61	72	74		F_8	F_{13}	F_{23}	F_{45}
Family type[a]					Number of families			
R	R	S	R	2	1		13	47
SEG	R	S	R				1	6
S	R	S	R		1		1	8
R	SEG	S	R					7
SEG	SEG	S	R				1	2
S	SEG	S	R					3
R	S	S	R					10
SEG	S	S	R					2
S	S	S	R	1				19
R	R	S	SEG				6	1
S	R	S	SEG		1			
R	SEG	S	SEG				1	
SEG	SEG	S	SEG				1	
R	S	S	SEG		2			1
SEG	S	S	SEG		1			
S	S	S	SEG		1	2		5
S	R	R	S	1	2		1	
R	SEG	R	S		1			
R	S	R	S		1			
S	S	R	S		7	1	6	
R	R	SEG	S		1			
S	S	SEG	S		1	4	3	1
R	R	S	S	2			3	
SEG	R	S	S				1	
R	SEG	S	S		1		1	1
SEG	SEG	S	S				1	
S	SEG	S	S					1
R	S	S	S	2	7	4	10	2
SEG	S	S	S		6	3	4	
S	S	S	S	20	98	118	78	16
Number of families				28	132	132	132	132
Number of different family types				6	16	6	17	17
Number of segregating families					15	9	20	30
Number of different segregating family types					9	3	10	12

[a] R, SEG, and S refer to resistant, segregating and susceptible families, respectively

[b] Data of Jackson et al. (1978)

Table 2. Frequencies of resistant (R), segregating (SEG), and susceptible (S) families (FAM)[a] and the frequencies of resistant and susceptible plants (P)[b] in the parents and four generations of CC II

Race	Disease reaction	Parental varieties	Generation							
			F_7	F_8	F_{15}	F_{13}	F_{25}	F_{23}	F_{47}	F_{45}
			P	FAM	P	FAM	P	FAM	P	FAM
40	R	0.18	0.05	0.11	0.08	0.03	0.18	0.26	0.85	0.52
	SEG	–	–	0.05	–	0.02	–	0.07	–	0.08
	S	0.82	0.95	0.84	0.92	0.95	0.82	0.67	0.15	0.40
61	R	0.25	0.11	0.04	0.12	0.00	0.23	0.20	0.78	0.47
	SEG	–	–	0.02	–	0.00	–	0.04	–	0.11
	S	0.75	0.89	0.94	0.88	1.00	0.77	0.76	0.22	0.42
72	R	0.04	0.02	0.08	0.01	0.01	0.01	0.05	0.04	0.00
	SEG	–	–	0.02	–	0.03	–	0.02	–	0.01
	S	0.96	0.98	0.90	0.99	0.96	0.99	0.93	0.96	0.99
74	R	0.11	0.17	0.02	0.14	0.00	0.28	0.12	0.92	0.79
	SEG	–	–	0.04	–	0.01	–	0.06	–	0.05
	S	0.89	0.83	0.94	0.86	0.99	0.72	0.82	0.08	0.16

[a] Families derived by self fertilization of single plants. The number of families scored was 132 in each generation

[b] Individual plants from population samples. Data on individual plants and parents are from Jackson et al. (1978)

The parents included three singly resistant and two doubly resistant family types. Several new singly and doubly resistant family types, which were not represented in the parents, appeared in various generations of CC II, including two family types resistant to race 72 (SSRS and RSRS). The singly and doubly resistant types tended to increase modestly in frequency over generations. The parents also included two triply resistant parents (RRSR) family types. One RRSR family was found in F_8 and none in F_{13} and 13 in F_{23}. However, by F_{45} RRSR had become the most common family type, represented by 47 of the 132 families (36%). No new triply resistant types, all of which would have involved the resistance to race 72 (SRRR, RSRR, RRRS), appeared in the population.

Family data of the type above permit us to test whether the changes in frequency of multiply resistant family types were significant and whether resistance to any one race is independent of resistance to other races. The number of possible family types is large, however, and to reduce the number of classes to a manageable level, we proceeded as follows. First, resistant and segregating families were combined into one class (called resistant), which reduces the number of classes to 16. Second the families were classified according to the number of races to which they were resistant (0, 1, 2, 3, and 4). These five classes include, respectively, 1, 8, 24, 32, and 16 of the original 81 family types.

Table 3 gives the observed and expected numbers of families resistant to zero to four races of scald in the parents and four generations of CC II. Comparisons of expected numbers (calculated from marginal totals) with observed numbers reveal that statistically significant changes occurred over generations in the frequencies of multiply resistant families. The main feature of the changes was a reversal of an excess of quadruply suceptible families in the earlier generations to an excess of resistant families, especially triply resistant families, in generation 45.

In testing whether resistance to the four races is random (independent) or nonrandom (correlated), observed numbers of families within each generation were compared with expected numbers calculated under the assumption that resistance to the four races is independent (Table 4). In calculating χ^2, classes were combined so that the expected number in any class was five or larger. There were only 28 parents; the expected number of multiply resistant family types was consequently small and the resulting reduction of number of classes precluded a meaningful test of significance. In generation 13, in which the general level of resistance was low, no multiply resistant families were expected and none were observed. However in generations F_8, F_{23} and F_{45} departures from independence were highly significant. This was due primarily to excess of triply resistant families and quadruply susceptible families. Single resistance was less frequent than expected in early generations, but this deficiency disappeared by F_{45}. Doubly resistant types were in small excess in F_8 but they had become less frequent than expected in genera-

Table 3. Observed (O), expected (E) and the difference between observed and expected numbers (O−E) of families resistant to 0, 1, 2, 3, or 4 races of scald. Expected numbers were calculated from marginal totals in testing for homogeneity over generations

N[a]		Parents		F_8		F_{13}		F_{23}		F_{45}	
		O	E	O	E	O	E	O	E	O	E
0		20	17	98	78	118	78	78	78	16	78
	(O−E)	3		20		40		0		−62	
1		3	4	22	21	14	21	23	21	28	21
	(O−E)	−1		1		−7		2		7	
2		3	2	9	10	0	10	8	10	25	10
	(O−E)	1		−1		−10		−2		15	
3		2	5	3	22	0	22	23	22	63	22
	(O−E)	−3		−19		−22		1		41	
4		0	0	0	0	0	0	0	0	0	0
	(O−E)	0		0		0		0		0	

[a] N is the number of races to which the family or parental variety is resistant

χ^2 for homogeneity = 230.0, $P < 0.001$. In calculating χ^2 classes were combined so that the expected number per class ≧ 5

tions F_{23} and F_{45}. It is therefore clear from these family data that reaction to certain races is associated with reaction to the other races.

Another way to test the family data for nonrandomness is to calculate pair wise correlations between resistance to the different races. The families were given values 0, 1, or 2 on the ordinal scale corresponding to S, SEG, R, and the product-moment correlation between resistance to pairs of races was calculated. This variable is analogous to the "number of principal alleles in the genotype" (Smouse and Neel 1977) but its meaning is less precise here because the present data represent phenotypic, not genotypic, classes. Nr^2 is distributed approximately as χ^2 when N is large and it consequently gives a test of significance, even though the primary variables are not normally distributed. It can be seen from Table 5 that significant positive associations occur between resistance to races 40, 61,

Table 4. Observed (O), expected (E) and the difference between observed and expected numbers (O−E) of families resistant to 0, 1, 2, 3, or 4 races of scald. Expected numbers were calculated assuming that resistance to single races is independent

N[a]		Parental varieties		F_8		F_{13}		F_{23}		F_{45}	
		O	E	O	E	O	E	O	E	O	E
0		20	16	98	88	118	118	78	51	16	3
	(O−E)	4		10		0		27		13	
1		3	10	22	38	14	14	23	57	28	29
	(O−E)	−7		−10		0		−34		−1	
2		3	2	9	6	0	0	8	21	25	61
	(O−E)	1		3		0		−13		−36	
3		2	0	3	0	0	0	23	3	63	39
	(O−E)	2		3		0		20		24	
4		0	0	0	0	0	0	0	0	0	0
	(O−E)	0		0		0		0		0	
χ^2		0.70		14.1		0.0		36.6		40.5	
Probability		N.S.		<0.001		N.S.		<0.001		<0.001	

[a] N is the number of races to which the parental variety or family is resistant

In calculating χ^2 classes were combined so that the expected number per class ≧ 5

Table 5. Pair wise correlations for resistance to races 40, 61, 72, and 74 in the parents and four generations of CC II

Generation		Race		
		61	72	74
P		0.663	– 0.105	0.378
F_8		0.257	0.009	0.257
F_{13}	40	–[a]	– 0.024	– 0.026
F_{23}		0.665	– 0.206	0.561
F_{45}		0.564	– 0.135	0.414
P			0.411	0.438
F_8			0.084	0.796
F_{13}	61		–[a]	–[a]
F_{23}			– 0.054	0.793
F_{45}			– 0.125	0.467
P				– 0.069
F_8				– 0.074
F_{13}	72			– 0.024
F_{23}				– 0.136
F_{45}				– 0.242

[a] Race 61 was invariant in F_{13}; consequently it was not possible to compute correlations with the other races

Correlations larger than 0.17 are significant at probability level 0.05

and 74 in the parental and in all generations except generation 13. Table 5 also shows that correlations between race 72 and the other races, although generally negative in sign, are usually nonsignificant. There was a positive and significant correlation between resistance to race 72 and race 61 in the parents but this correlation had become negative and was no longer significant in the later generations.

Discussion

In most studies of the inheritance of reaction to scald disease, resistance has been found to be governed by alleles at single loci. Alleles for resistance have usually been found to be dominant to those for susceptibility but in a few cases resistance alleles have been either partly dominant or recessive. One case of recessive epistasis has been reported (Baker and Larter 1963). Several of the parents of CC II are known to carry specific genes for resistance to scald. 'Atlas' carries the gene *Rh2* (Dyck and Schaller 1961 a). 'Trebi' carries a dominant resistance allele of the gene *Rh4*, which is located on chromosome 3 (Dyck and Schaller 1961 b; Riddle and Briggs 1950), as well as a recessive resistance allele of an unnamed gene (Riddle and Briggs 1950). 'Trebi' possibly also carries a resistance allele of gene *Rh3*. *Rh3* has been identified in the cross 'Wisconsin Winter' × 'Glabron', both of which are parents of CC II. Habgood and Hayes (1971) reported that resistance is partially dominant to susceptibility for *Rh3*.

Jackson and Webster (1976 a) tested 'Atlas', 'Trebi' and 'Wisconsin Winter' × 'Glabron' against races 40, 61, 72, and 74. These materials were found to be susceptible to all four races except that 'Trebi' is resistant to race 40 and 'Atlas' to races 61 and 72. The data of Jackson et al. (1978) show that races 40, 61, 72, and 74 possess virulence alleles that are able to overcome all of the known resistance genes in the parents of CC II. However it is obvious that the parents of CC II must carry genes for resistance in addition to those that have been reported in the literature because at least one parental variety was resistant to each of the races.

In the present study classification of families was into resistant, segregating and susceptible categories. Family sizes were too small to justify formal Mendelian analyses of observed ratios in segregating families. However the data reveal an interesting feature of inheritance at the population level: the frequency of segregating families, even though underestimated in this experiment, was high in all generations and their frequency increased in the later generations (the observed proportions of segregating families in generations F_8, F_{13}, F_{23}, and F_{45} were 11, 9, 15, and 23%, respectively). Similar high frequencies of segregating families have been found in barley Composite Cross V by Jackson et al. (in preparation). In a population such as CC II, in which the mating system is one of more than 99% of self fertilization, it is expected that inbreeding equilibrium will have been closely approached within 8 generations after synthesis of the population, and that the frequency of heterozygotes at any locus will thereafter fluctuate only narrowly about the equilibrium point. It is also expected that, at inbreeding equilibrium, the proportion of heterozygotes will be low, even when mechanisms that promote heterozygosity (e.g. heterozygote advantage) are taken into account (Allard et al. 1968). Kahler and Allard (in preparation) have established that equilibrium heterozygosity levels for electrophoretic markers in CC II fall in the range of one to two percent. The high frequency of families segregating for disease reaction observed in CC II is therefore not consistent with single-locus inheritance of resistance. This conclusion is supported by the observation that many families segregated simultaneously for resistance to two races and one family in F_{23} segregated simultaneously for resistance to three races. Whatever the genetic basis of resistance in the parental varieties, it is clear that the segregation and recombination which have occurred in CC II have given rise to numerous novel multiresistant genotypes.

In total, 30 different family types were found among the parents and the four generations studied. The pattern of multiple resistance was nonrandom: in most generations triply resistant and completely susceptible families were more frequent than expected on the basis of resistance to individual races. Another finding was that resistance to races 40, 61, and 74 was positively correlated in pair wise comparisons. Resistance to race 72, on the other hand, was independent of resistance to the other races. Jackson et al. (in preparation) have found similar patterns of correlation in barley Composite Cross V.

Several hypotheses can be formulated to account for the observed correlations. The simplest and most obvious hypothesis is that resistance to races 40, 61, and 74 is governed by the same locus. The existence of parents and families that are singly, doubly, or triply resistant, as well as families that are segregating for resistance to 1, 2 or all 3 races simultaneously is, however, inconsistent with this hypothesis. Another possible hypothesis is that the two RRSR parents carry a tightly linked cluster of three or more genes for resistance. With tight linkage little or no recombination is expected following the initial crossing cycle, nor as a result of the low level of intercrossing which occurs in each of the later generations. It is therefore expected that the initial correlation between resistance to the three races would decline very slowly over generations. However the corelations between resistance to races 40, 61, and 74 did not follow this pattern for any of the pairs of races. The correlation between resistance to races 40 and 61, which was high in the parents, fell off in F_8 but increased again to high levels in the later generations. For pairs 40 and 74 the correlations decreased from parents to F_8 to F_{13} but then increased again, whereas for pair 61 and 74 it was higher in the middle generations than in the parents or generation 45. The hypothesis of tight linkage is thus also not consistent with the observations. Still another hypothesis is that the correlations are due to epistatic selection, which can be a powerful force in generating and developing gametic disequilibrium between favored combinations at different genetic loci in inbreeding populations (Clegg et al. 1972; Weir et al. 1972, 1974). The present data do not allow decisive tests of this hypothesis. Formal genetic studies of the scald reaction of families derived from appropriate hybrids have been initiated to determine the basis of the observed correlations.

Acknowledgment

This research was supported in part by N.S.F. Grant DEB-7802046 and SEA-USDA Grant 79-59-2063-1-1-204-1.

Literature

Allard, R.W.; Jain, S.K.; Workman, P.L. (1968): The genetics of inbreeding populations. Adv. Genet. **14**, 55–131

Baker, R.J.; Larter, E.N. (1963): The inheritance of scald resistance in barley. Can. J. Genet. Cytol. **5**, 445–449

Clegg, M.T.; Allard, R.W.; Kahler, A.L. (1972): Is the gene the unit of selection? Evidence from two experimental plant populations. Proc. Nat. Acad. Sci. USA **69**, 2474–2478

Dyck, P.L.; Schaller, C.W. (1961a): Inheritance of resistance in barley to several races of the scald fungus. Can. J. Genet. Cytol. **3**, 153–164

Dyck, P.L.; Schaller, C.W. (1961b): Association of two genes for scald resistance with a specific barley chromosome. Can. J. Genet. Cytol. **3**, 165–169

Habgood, R.M.; Hayes, J.D. (1971): The inheritance of resistance to *Rhynchosporium secalis* in barley. Heredity **27**, 25–37

Harlan, H.V.; Martini, M.L. (1929): A composite hybrid mixture. J. Am. Soc. Agron. **21**, 487–490

Jackson, L.F.; Webster; R.K. (1976a): Race differentiation, distribution, and frequency of *Rhynchosporium secalis* in California. Phytopathology **66**, 719–725

Jackson, L.F.; Webster, R.K. (1976b): The dynamics of a controlled population of *Rhyncosporium secalis,* changes in race composition and frequencies. Phytopathology **66**, 726–728

Jackson, L.F.; Kahler, A.L.; Webster, R.K.; Allard, R.W. (1978): Conservation of scald resistance in barley composite cross populations. Phytopathology **68**, 645–650

Jackson, L.F.; Webster, R.K.; Kahler A.L.; Allard, R.W. Genetic analysis of changes in scald resistance in barley Composite Cross V. Phytopathology (in preparation)

Jackson, M. (1979): Studies on the nature of host resistance to barley scald and its influence on pathogenic complexity in *Rhynochosporium secalis.* Ph. D. Thesis, University of California, Davis, California

Kahler, A.L.; Allard, R.W. Evidence for coadaptation in Composite Cross II, an experimental population of barley. (in preparation)

Riddle, O.C.; Briggs, F.N. (1950): Inheritance of resistance to scald in barley. Hilgardia **20**, 19–27

Smouse, P.; Neel, J.V. (1977): Multivariate analysis of gametic disequilibrium in the Yanomana. Genetics **85**, 733–752

Weir, B.S.; Allard, R.W.; Kahler, A.L. (1972): Analysis of complex allozyme polymorphisms in a barley population. Genetics **72**, 505–523

Weir, B.S.; Allard, R.W.; Kahler, A.L. (1974): Further analysis of complex polymorphisms in a barley population. Genetics **78**, 911–919

Received September 17, 1981
Communicated by H. F. Linskens

Dr. O. Muona
Prof. Dr. R. W. Allard
Department of Genetics
University of California
Davis, Calif. 95616 (USA)

Dr. R. K. Webster
Department of Plant Pathology
University of California
Davis, Calif. 95616 (USA)