

Genetic Analysis of Changes in Scald Resistance in Barley Composite Cross V

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ABSTRACT

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Families derived by self-pollinating a random sample of plants from four generations (F₅, F₁₂, F₂₃, and F₃₂) of barley Composite Cross V were inoculated with isolates of races 40, 61, 72, 74 of *Rhynchosporium secalis* and scored as resistant, susceptible or segregating for scald disease reaction. This population, which was synthesized from intercrosses among 30 barley cultivars, has been grown annually at Davis, CA, under standard agricultural conditions without conscious selection. Many different family types were found in all generations, including numerous segregating families. A test for randomness showed that families susceptible to all four

races and families resistant to three races were more frequent 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 the other races. Possible reasons for these correlations are discussed. The appearance, disappearance, and reappearance of different family types in successive generations and the changes in frequency of multiply resistant families appear to represent responses to biological stress caused by the variable and shifting pathogen population.

Additional key words: conservation of resistance, inheritance of resistance, plant breeding.

In a recent paper (9), we reported the results of assaying individual plants in several generations of barley (*Hordeum vulgare* L.) Composite Cross V (CC V) for reaction to four isolates of *Rhynchosporium secalis* (Oud.) Davis. These assays showed little change in the frequency of individuals carrying specific resistance to scald disease over the 27-generation span that was studied. The study reported in this paper differs from the earlier study in that families derived from selfed plants were assayed for reaction to the same four isolates of *R. secalis*. Thus, the reaction of each family to the different isolates could be determined and families that were segregating for resistance could be distinguished from families composed of only resistant or only susceptible plants. In this paper we report the results of genetic analyses of these family data.

MATERIALS AND METHODS

The synthesis and methods of growing CC V were described by Suneson (14). The materials of the present study were derived from 250 seeds each randomly taken from plants of generations F₅, F₁₂, F₂₃, and F₃₂. These seeds were germinated in metal flats in the greenhouse and the resulting 2-wk-old seedlings (224 of F₅, 222 of F₁₂, 214 of F₂₃, and 223 of F₃₂) were inoculated with isolates (ATCC 34256, ATCC 34277, ATCC 34288, and ATCC 34290) of races 40, 61, 72, and 74, respectively (9). All seedlings (no individuals were lost in this phase of the study) were transplanted into pots and grown to maturity in the greenhouse. Seeds were harvested from each adult plant. Four 12-seed lots were made up from each plant and planted in metal flats. When the seedlings reached the one-and-one-half to three-leaf stage, each lot was inoculated with one of the four isolates. Inoculations were made under standardized conditions as described by Jackson and Webster (10). The seedlings were scored for disease reaction 2 wk later. Scoring was on a scale

of zero to four: 0 = no visible symptoms; 1 = very small lesions confined to leaf margins; 2 = small lesions not confined to leaf margins; 3 = large coalescing lesions involving a majority of the leaf area; 4 = total collapse of the leaf. Plants scored 0, 1, and 2 were considered resistant reactions, and those scored three and four were considered susceptible reactions. Families that included both resistant and susceptible individuals were recorded as segregating for reaction to the isolate involved. Because the family sizes were small, the proportion of segregating families was almost certainly underestimated.

RESULTS

Table 1 lists the family types observed in four generations of CC V and the family types of the parents as reported by Jackson et al (9). Table 2 gives the frequencies of resistant (R), segregating (SEG), and susceptible (S) families observed in the present study and the frequencies of resistant and susceptible individuals reported by Jackson et al (9). Because resistance is usually dominant to susceptibility (3,5-8,13), most segregating families probably arose from resistant plants. Therefore, the frequencies of susceptible families in the present study can be compared without risk of serious error to the frequencies of susceptible individuals in the study of Jackson et al (9). The frequencies of susceptible families in the present study are similar to the frequencies of susceptible individuals in the earlier study (Table 2).

There are three possible family types (R, SEG, S) for each of the four races and thus 3⁴ = 81 possible family types in total. Among the 81 possible family types, 30 were observed, including six in the parents and much larger numbers in generations F₅, F₁₂, F₂₃, and F₃₂ (22, 16, 15, and 11, respectively) (Table 1). Family type SSSS (susceptible to all four races) was the most common type observed in the parents (70%) and in generations F₅, F₁₂, F₂₃, and F₃₂ (65, 73, 89, and 87%, respectively). The parents included two singly resistant (SRSS and SSSR) and two doubly resistant (RRSS and SRRS) types. One of the singly resistant types (SSSR) was not observed in any of the later generations. Neither of the doubly resistant types was observed in later generations. The parents also included one triply resistant type (RRSR). This type was present in

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about 15% of the individuals in generations F₅ and F₁₂, but it decreased to a frequency of less than 1% in generation F₂₃, and then increased to about 3% frequency in generation F₃₂. Many family types not present in the parents appeared in the four generations of CC V that were studied. The most interesting among these novel types was the quadruply resistant type, RRRR, which appeared in generation F₅, but was not observed in later generations.

Data of the type described above permit testing of whether significant changes occurred in the frequency of different types in successive generations. However, the number of possible family types is large 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 (denoted resistant), which reduced 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, or 4), which reduced the number of classes to five.

Comparisons of observed and expected numbers of families resistant to races 0 to 4 show that statistically significant changes occurred over generations in the frequency of multiply resistant families (Table 3). The main feature of the changes was the development of an excess of quadruply susceptible families and a concomitant decrease in the frequency of multiply resistant families in the later generations.

To test whether resistance to the four races is random (independent) or nonrandom (correlated), observed numbers of families resistant to 0 to 4 races within each generation were

TABLE 1. Observed numbers of scale resistant (R), segregating (SEG), and susceptible (S) families in the parents and four generations of barley Composite Cross V

Family type regarding races:				Number of parent cultivars	Number of families in generations:			
40	61	72	74		F ₅	F ₁₂	F ₂₃	F ₃₂
R	S	S	S		1	2	1	4
S	R	S	S	1	3	2		3
S	S	R	S		1		4	
S	S	S	R	2				
R	R	S	S	2		1	1	
R	S	R	S		1			
R	S	S	R					2
S	R	R	S	1	6	1		
R	R	S	R	3	34	32	1	6
R	R	R	R		3			
S	S	S	S	21	145	162	190	193
SEG	R	R	R		1			
SEG	S	S	S		1	5	3	10
SEG	R	S	S				1	
SEG	S	S	R				1	
R	SEG	S	R		1	2	5	1
S	SEG	R	S		1	1	2	1
S	SEG	S	S		5	5		1
R	SEG	S	S					1
S	S	SEG	S		6	1	1	
S	R	SEG	S		1			
R	R	S	SEG		1	1		
R	S	S	SEG				1	
S	S	S	SEG		2	1		
SEG	SEG	S	S		1			
SEG	S	S	SEG			2	1	
S	SEG	SEG	S		1	1		
S	SEG	S	SEG		1	1		
R	SEG	S	SEG		2	1		1
SEG	SEG	S	SEG		6	3		
Totals								
Number of families				30	224	222	214	223
Number of different family types				6	22	16	15	11
Number of segregating families					30	22	17	15
Number of different segregating family types					14	10	10	6

compared with expected numbers calculated assuming that resistance to the four races is independent (Table 4). Statistically significant departures from independence were observed in all generations. These departures featured an excess of quadruply susceptible families, deficiencies of singly and/or doubly resistant families, and an excess of triply resistant families in all generations. These departures, which were large in the earlier generations, all moderated in the later generations. Thus, although reaction to different races was associated with reaction to other races, the extent of the association changed in successive generations.

Another way to test the family data for randomness is to calculate pairwise correlations between resistance to the four races. The families were given values of 0, 1, or 2 (corresponding to S, SEG, or R, respectively) and product moment correlations were calculated between resistance to pairs of races within each generation. Nr² is distributed approximately as χ^2 when N is large

TABLE 2. Frequencies of scald resistant (R), segregating (SEG), and susceptible (S) families (FAM)^a and plants (P)^b in the parents and four selected generations of barley Composite Cross V

Race	Disease reaction	Parental cultivars	Generation							
			F ₅		F ₁₂		F ₂₃		F ₃₂	
			P	FAM	P	FAM	P	FAM	P	FAM
40	R	0.17	0.25	0.19	0.19	0.18	0.10	0.04	0.12	0.07
	SEG	0.04	...	0.04	...	0.03	...	0.04
	S	0.83	0.75	0.77	0.81	0.78	0.90	0.93	0.88	0.89
61	R	0.23	0.14	0.22	0.16	0.17	0.10	0.01	0.14	0.04
	SEG	0.08	...	0.06	...	0.04	...	0.02
	S	0.77	0.86	0.70	0.84	0.77	0.90	0.95	0.86	0.94
72	R	0.03	0.05	0.06	0.07	0.01	0.06	0.03	0.03	0.01
	SEG	0.04	...	≤0.01	...	0.01	...	≤0.01
	S	0.97	0.95	0.90	0.93	0.99	0.94	0.96	0.97	0.98
74	R	0.17	0.16	0.17	0.14	0.15	0.03	0.03	0.04	0.04
	SEG	0.05	...	0.04	...	0.01	...	≤0.01
	S	0.83	0.84	0.78	0.86	0.81	0.97	0.96	0.96	0.96

^aFamilies derived by self-fertilization of single plants. Numbers of families scored were 224, 222, 214, and 223 in F₅, F₁₂, F₂₃, and F₃₂, respectively.

^bIndividual plants from population samples. Data on individual plants and parents are from Jackson et al (9).

TABLE 3. Observed, expected, and the difference between observed and expected numbers of parents and families of barley Composite Cross V resistant to races 0, 1, 2, 3, or 4 of *Rhynchosporium secalis*. Expected numbers were calculated from marginal totals in testing for homogeneity over generations

N ^a		Parents	F ₅	F ₁₂	F ₂₃	F ₃₂
0	Observed	21	145	162	190	193
	Expected	24	174	173	166	174
	Difference	-3	-29	-11	24	19
1	Observed	3	19	15	10	18
	Expected	2	16	16	16	16
	Difference	1	3	-1	-6	2
2	Observed	3	12	6	8	4
	Expected	1	8	8	8	8
	Difference	2	4	-2	0	-4
3	Observed	3	44	39	6	8
	Expected	3	25	25	23	24
	Difference	0	19	14	-17	-16
4	Observed	0	4	0	0	0
	Expected	0	1	1	1	1
	Difference	0	3	-1	-1	-1

^aN is the number of races to which the cultivar or family is resistant. χ^2 for homogeneity = 71.8, $P \leq 0.001$. In calculating χ^2 , classes were combined so that the expected numbers per class ≥ 5 .

and it consequently gives a test of significance, even though the primary variables are not normally distributed. Correlations were positive and significant between races 40, 61, and 74 and in the parental and all other generations (Table 5). Correlations between races 61 and 72, although smaller, were consistently positive and also significant in all generations except F₁₂. Correlations between race 72 and races 40 and 74, although generally negative in sign, were consistently not statistically significant.

DISCUSSION

In most studies of the inheritance of reaction to scald disease, resistance proves to be governed by alleles at single loci. Alleles for resistance are usually dominant to alleles for susceptibility, but in a few cases resistance alleles have been partly dominant or recessive, and one case of recessive epistasis has been reported (3,5-8,13). Several of the parents of CC V carry specific alleles for resistance to scald. Thus, Atlas carries *Rh*₂ (5), Trebi carries *Rh*₄ (5) a recessive allele of an unnamed gene (13), and possibly a resistance allele at locus *Rh*₃. Jackson and Webster (10) tested Atlas and Trebi against races 40, 61, 72, and 74 and found that Atlas is resistant to races 61 and 72 and Trebi is resistant to race 40. The data of Jackson et al (9) show that races 40, 61, 72, and 74 carry virulence alleles that are able to overcome all of the known resistance alleles in the parents of CC V. However, because at least one parental cultivar was resistant to each of the races it is evident that the parents of CC V carry resistance alleles in addition to those that have been reported in the literature. It is also evident that CC V is complex with respect to alleles for resistance to scald.

Studies of the pathogen population indicate that its genetic composition is complex with respect to virulence. At least 75 different physiological races occur in the Sacramento and San Joaquin valleys of California (10), and large changes in the racial composition of the population have occurred over time (M. Jackson, unpublished). The racial composition of populations of *R. secalis* can change rapidly in controlled greenhouse experiments (11). Thus, both CC V and *R. secalis* represent highly variable populations with respect to alleles for resistance and virulence, respectively.

In the present study, families were classified as resistant, segregating, or susceptible to four races of *R. secalis*. Family sizes were too small to permit 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 it was underestimated in this experiment, was high in all generations. Similar high frequencies of segregating families have been found in barley CC II by Muona et al (12). In a population such as CC V, in which the mating system is one of more than 99% of self-fertilization, the population is expected to closely approach inbreeding equilibrium within six to eight generations after its synthesis. Thereafter, the frequency of heterozygotes at any locus is expected to fluctuate only narrowly about the equilibrium point. At inbreeding equilibrium, the proportion of heterozygotes is expected to be low, even when mechanisms that promote heterozygosity (eg, heterozygote advantage) are taken into account (1). Allard et al (2) showed that equilibrium heterozygosity levels for electrophoretic markers in CC V fall in the range of 1-2%. The high frequency of families segregating for disease reaction observed in CC V is therefore not consistent with single-locus inheritance of resistance. This conclusion is supported by the observation that four families segregated for resistance to two races and one family segregated for resistance to three races. Regardless of the genetic basis of resistance in the parental cultivars, it is clear that the segregation and recombination that have occurred in CC V have given rise to numerous novel multiply resistant 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 completely susceptible and triply resistant families were more frequent than expected on the basis of resistance to individual races. In addition, resistance to races 40, 61, and 74 was positively correlated in pairwise

comparisons. Resistance to race 72, on the other hand, was independent of resistance to the other races. Muona et al (12) found similar patterns of correlation in barley Composite Cross (CC) II.

Several hypotheses may 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 one, two, or all three races simultaneously is, however, inconsistent with this hypothesis. Another hypothesis is that the three 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 that occurs in each of the later generations. Therefore, the initial correlation between resistance to the three races is

TABLE 4. Observed, expected, and difference between observed and expected numbers of parents and families of barley Composite Cross V resistant to races 0, 1, 2, 3, or 4 of *Rhynchosporium secalis*. Expected numbers were calculated assuming that resistance to single races is independent

N ^a		Parents	F ₅	F ₁₂	F ₂₃	F ₃₂
0	Observed	21	145	162	190	193
	Expected	15	85	107	174	177
	Difference	6	60	55	16	16
1	Observed	3	19	15	10	18
	Expected	12	95	88	37	43
	Difference	-9	-76	-73	-27	-25
2	Observed	3	12	6	8	4
	Expected	3	38	24	3	3
	Difference	0	-26	-18	5	1
3	Observed	3	44	39	6	8
	Expected	0	6	3	0	0
	Difference	3	38	36	6	8
4	Observed	0	4	0	0	0
	Expected	0	0	0	0	0
	Difference	0	4	0	0	0
X ²		4.8	414.9	100.8	5.6	7.0
Probability		≤0.05	≤0.001	≤0.001	≤0.02	≤0.01

^aN is the number of races to which the parental cultivar or family is resistant. In calculating χ^2 classes were combined so that the expected number per class ≥ 5 .

TABLE 5. Pairwise correlations for resistance to races 40, 61, 72, and 74 of *Rhynchosporium secalis* in the parents and four generations of barley Composite Cross V

Generation	Race	Race		
		61	72	74
P	40	0.811	-0.083	0.520
F ₅		0.801	0.041	0.939
F ₁₂		0.872	-0.058	0.924
F ₂₃		0.673	-0.050	0.794
F ₃₂		0.525	-0.023	0.734
P	61		0.337	0.388
F ₅			0.298	0.831
F ₁₂			0.120	0.893
F ₂₃			0.187	0.541
F ₃₂			0.145	0.677
P	72			-0.083
F ₅				0.028
F ₁₂				-0.053
F ₂₃				-0.041
F ₃₂				-0.014

Values larger than 0.138 are statistically significant, $P = 0.05$.

expected to decline very slowly in successive generations. However, the correlations 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 was high in the parents, but fell off rather rapidly in successive generations. For races 40 and 74, and 61 and 74, the correlations were low in the parents, increased in the middle generations, and fell off again in the later generations. Thus, the hypothesis of tight linkage is 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 (4, 15, 16). 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.

The presence of many different types of families, including large numbers of segregating families, in each generation shows that significant variability in disease resistance is maintained in CC V. The appearance, disappearance, and reappearance of different family types from generation to generation appear to represent dynamic adaptable responses to a variable and shifting pathogen population.

LITERATURE CITED

1. Allard, R. W., Jain, S. K., and Workman, P. L. 1968. The genetics of inbreeding populations. *Adv. Genet.* 14:55-131.
2. Allard, R. W., Kahler, A. L., and Weir, B. S. 1972. The effect of selection on esterase allozymes in a barley population. *Genetics* 72:489-503.
3. Baker, R. J., and Larter, E. N. 1963. The inheritance of scald resistance

in barley. *Can. J. Genet. Cytol.* 5:445-449.

4. Clegg, M. T., Allard, R. W., and 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.
5. Dyck, P. L., and Schaller, C. W. 1961. Inheritance of resistance in barley to several physiologic races of the scald fungus. *Can. J. Genet. Cytol.* 3:153-164.
6. Dyck, P. L., and Schaller, C. W. 1961. Association of two genes for scald resistance with a specific barley chromosome. *Can. J. Genet. Cytol.* 3:165-169.
7. Habgood, R. M. 1974. The inheritance of resistance to *Rhynchosporium secalis* in some European spring barley cultivars. *Ann. Appl. Biol.* 77:191-200.
8. Habgood, R. M., and Hayes, J. D. 1971. The inheritance of resistance to *Rhynchosporium secalis* in barley. *Heredity* 27:25-37.
9. Jackson, L. F., Kahler, A. L., Webster, R. K., and Allard, R. W. 1978. Conservation of scald resistance in barley composite cross populations. *Phytopathology* 68:645-650.
10. Jackson, L. F., and Webster, R. K. 1976. Race differentiation, distribution, and frequency of *Rhynchosporium secalis* in California. *Phytopathology* 66:719-725.
11. Jackson, L. F., and Webster, R. K. 1976. The dynamics of a controlled population of *Rhynchosporium secalis*, changes in race composition and frequencies. *Phytopathology* 66:726-728.
12. Muona, O., Allard, R. W., and Webster, R. K. 1982. Evolution of resistance to *Rhynchosporium secalis* (Oud.) Davis in barley Composite Cross II. *Theor. Appl. Genet.* 61:209-214.
13. Riddle, O. C., and Briggs, F. N. 1950. Inheritance of resistance to scald in barley. *Hilgardia* 20:19-27.
14. Suneson, C. A. 1969. Registration of barley composite crosses. *Crop Sci.* 9:395-396.
15. Weir, B. S., Allard, R. W., and Kahler, A. L. 1972. Analysis of complex allozyme polymorphisms in a barley population. *Genetics* 72:505-523.
16. Weir, B. S., Allard, R. W., and Kahler, A. L. 1974. Further analyses of complex polymorphisms in a barley population. *Genetics* 78:911-919.