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Evolution of resistance to scald, powdery mildew, and net blotch in barley composite cross II populations

M. A. Saghai Maroof[1], R. K. Webster[2] and R. W. Allard[1]

[1] Department of Genetics, [2] Department of Plant Pathology; University of California, Davis, CA 95616, USA

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Summary. Progenies of barley plants taken at random from generations F_8, F_{13}, F_{23}, and F_{45} of Composite Cross II were tested for reaction to three barley pathogens, *Helminthosporium teres, Erysiphe graminis,* and *Rhynchosporium secalis* (four races). The frequency of families resistant to each of the three pathogens (excepting one race of *R. secalis*) increased from early to later generations. Many families carrying combinations of multiple resistance not observed among the parents were found in the later generations; one-half of the parents were susceptible to all three diseases but only 4% of the families in generation F_{45} were of this phenotype. The frequency of multiply resistant families also increased sharply over generations. Among the parents the greatest concentration of resistant reactions observed was triple resistance: 14% were triply resistant; none were quadruply resistant; nor were any resistant to all three pathogens. In generation F_{45} 36% of the families were triply resistant, 32% were quadruply resistant and 6% were resistant in five of the six disease reactions. The value of later generations of Composite Cross II for breeding against pathogens thus appears to have been increased by recombination and natural selection. Significant positive correlations were found for resistance to races 40, 61, and 74 of *R. secalis* whereas correlations between resistance to *R. secalis, E. graminis,* and *H. teres* were generally nonsignificant. Use of these multiply resistant genotypes in breeding is thus unlikely to be hindered by negative associations between resistance to the three diseases.

Key words: Disease resistance – Genetic variability – Plant breeding

Introduction

Scald, caused by *Rhynchosporium secalis* (Oud.) Davis, is an important disease of barley (*Hordeum vulgare* L.). Losses in grain yields as high as 35% and 70% have been reported in California (Schaller 1951) and Australia (Ali et al. 1976), respectively. Powdery mildew, caused by *Erysiphe graminis* DC. f. sp. hordei Em. Marchal, and net blotch, caused by *Helminthosporium teres* (Died.) Drechsl., also reduce grain yield in barley. There are many different races of each of these three diseases (scald-Jackson and Webster 1976; powdery mildew-Moseman 1966; net blotch-Singh 1962), and barley is genetically variable for resistance to these diseases. Several loci governing resistance to *H. teres* have been identified on chromosomes 2, 3, and 5, as have 17 loci governing resistance to *R. secalis,* most of which are on chromosome 3, and 10 loci governing resistance to *E. graminis* most of which are on chromosome 5 (Bockelman et al. 1977; Webster et al. 1980; Moseman 1966).

Successes in controlling these diseases by the development of resistant cultivars have been short-lived: new sources of resistance have been identified and incorporated into commercial cultivars, only to succumb quickly to new races of the pathogens. That genetic variability, including genetic variability for disease resistance, is present and conserved in composite crosses of barley is well known (Allard and Jain 1962; Jackson et al. 1978). The regularly low incidence of disease that has been observed in later generations of these populations has led us to this investigation of their potential for providing stable resistance to variable pathogens.

Barley Composite Cross II (CC II) was synthesized in 1929 by making all 378 possible crosses among 28 barley varieties drawn from the major barley-growing areas of the world; F_1 hybrid seeds were pooled and planted (Harlan and Martini 1929). Since then, the population has been advanced one generation each year under standard agricultural conditions without conscious selection at Davis, California. In addition, the older generations have been advanced one generation every ten years or so to maintain viable seeds. These seed collections thus provide materials that permit study of evolutionary changes over long sequences of generations.

Some of the parental varieties of the composite crosses have alleles conferring resistance to these diseases. For example, 'Atlas' and 'Trebi' have alleles for resistance to *R. secalis* and 'Algerian' has alleles for resistance to *H. teres* and *E. graminis*. Jackson et al. (1978) determined the proportion of plants resistant to scald in early, intermediate, and late generations of three barley composite crosses, including generations 7, 15, 25, and 47 of CC II. They infected plants with isolates of four races of *R. secalis* and found that in CC II resistance to three of the four races increased over time. Muona et al. (1982) conducted studies to determine whether the observed changes in resistance to different races are correlated. They used the same four races of scald to inoculate generations 8, 13, 23, and 45 of CC II. Their data showed that some plants were multiply resistant, and they found increasing positive correlations among the three resistances that increased over generations.

We report here the results of a further study of multiple resistance in the four generations of CC II that were studied by Muona et al. Resistance to the pathogens *E. graminis* and *H. teres* as well as to the same four races of *R. secalis* was determined. The experiments were designed to address the questions whether resistance to these diseases increased, whether the frequency of plants resistant to more than one disease increased, and whether the observed resistances were independent or correlated.

Materials and methods

Fifty seeds chosen at random from each of generations 8, 13, 23, and 45 of CC II were sown. The resulting 200 plants (50 per generation) were grown to maturity; seed was collected from each plant and kept separate as a single "family". Five lots of 10–14 seeds from each of the 200 families, and also from each of the 28 parental varieties, were sown in flats in a greenhouse for tests of susceptibility to powdery mildew and to the four races of scald.

Treatments were applied when the seedlings were at the two-leaf stage (about two weeks after sowing). The flats assigned to powdery-mildew treatment were uniformly dusted with *E. graminis* conidia from heavily infected barley plants from the field. These plants were left in the greenhouse. The flats assigned to the scald treatments were moved into four isolated compartments in a controlled-environment chamber. The flats in each of the compartments were inoculated (50 ml per flat) with a spore suspension (2×10^5 spores per ml) of one of the four races (40, 61, 72, or 74) of *R. secalis* with a DeVilbiss Adjustable Tip Atomizer 15 attached to an air hose. After 3 h, the humidity of the chambers was brought to 100% to provide the optimal environment for growth of the spores. The flats were returned to a different greenhouse two days later. After two weeks, plants were rated for scald, and after three weeks, for powdery mildew. Table 1 gives the systems of rating.

Relying upon natural sources of infection, we studied net-blotch resistance in a field experiment. Forty seeds from each of the same 200 families and 28 parental varieties were sown in rows of 10 seeds each; the rows were arranged in the field in a randomized complete block design with four replications. Plants of the susceptible cultivar 'Atlas' were interspersed to serve as "spreaders" and also as indicators of the distribution

Table 1. Systems of rating disease reaction to the three pathogens

Score or phenotype	Disease		
	Scald	Powdery mildew	Net blotch
0	No symptoms	No symptoms	No symptoms
1	Very small lesions confined to leaf margins	Flecks and no sporulation	Small purple spots, no chlorosis or netting
2	Small lesions not confined to leaf margins	Lesions and no sporulation	Large, brown, scattered spots, some netting and no chlorosis
3	Large lesions	Fewer than 10 sporulating lesions per leaf	Brown, small and large lesions, some chlorosis and netting, some lesions elongating, both upper and lower leaves affected
4	Total collapse of leaf	More than 10 sporulating lesions per leaf	Lesions coalescing, at least half of leaf surface affected, chlorosis and lesions on spikes
5		Abundant lesions coalesced with profuse sporulation	
Resistant	0–2	0–3	0–2
Susceptible	3–4	4–5	3–4

Table 2. Frequencies of families resistant (Res), segregating (Seg), and susceptible (Sus) to the three pathogens in four generations of CC II

Generation					Race of *R. secalis*											
	H. teres		*E. graminis*		40			61			72			74		
	Res	Sus	Res	Sus	Res	Seg	Sus	Res	Seg	Sus	Res	Seg	Sus	Res	Seg	Sus
8	0.14	0.86	0.20	0.80	0.02	0.10	0.88	0.04	0.08	0.88	0.08	0.00	0.92	0.02	0.00	0.98
13	0.08	0.92	0.29	0.71	0.04	0.06	0.90	0.00	0.04	0.96	0.02	0.00	0.98	0.02	0.04	0.94
23	0.18	0.82	0.13	0.87	0.16	0.00	0.84	0.08	0.06	0.86	0.04	0.04	0.92	0.08	0.04	0.88
45	0.40	0.60	0.29	0.71	0.80	0.08	0.12	0.34	0.28	0.38	0.02	0.04	0.94	0.72	0.14	0.14

and severity of infection. *H. teres* infection on 'Atlas' was severe and uniform throughout the plot. The experimental plants were rated for their reaction to *H. teres* at heading time according to the system in Table 1.

Results and discussion

As Table 2 shows, resistance to all three pathogens increased over generations; only resistance to race 72 of *R. secalis* did not show much change. The rate of increase in frequencies of plants resistant to races 40, 61, and 74 of *R. secalis* was greatest between generations 23 and 45. These findings, which are consistent with those of Jackson et al. (1978) and Muona et al. (1982), extend their results in showing that the frequencies of plants resistant to *H. teres* and *E. graminis* as well as to *R. secalis* increased over generations.

Combinations of resistance or susceptibility in single families are of particular interest. If reaction to each pathogen is classified as either resistant or susceptible, there are $2^6 = 64$ possible family types. We observed a total of 31 different family types in the course of our study, 11 of which were observed in the parental varieties. Only three of the 11 parental types were not observed in our samples from the later generations. In the F_8 seven of the parental family types were observed and in each of the other three generations five of the parental family types were observed. Three new family types were observed in the F_8, four in the F_{13}, six in the F_{23}, and seven in the F_{45}.

Many new combinations of resistant reactions not observed in the parental or early generations appeared in later generations. The phenotype for joint susceptibility to *H. teres, E. graminis,* and all four races of *R. secalis* was the most common one among the parents (50% of the plants were of this phenotype). Its frequency was drastically reduced by generation F_{45} in which only 4% of the families were of this phenotype. In contrast, the frequency of multiply resistant types increased over generations, as Table 3 shows. Among single lines of the parental varieties, the greatest combination of resistant reactions observed was triple resistance; 14% were triply resistant; none were quadruply resistant, nor were any resistant to all three pathogens. In the F_{45} 36% of the families were triply resistant, 32% were quadruply resistant, and 6% were resistant in five out of their six reactions. Only one parental type was resistant both to *H. teres* and to one of the races of *R. secalis* (race 40). In generation F_{45} 20 families were resistant both to *H. teres* and to at least one race of *R. secalis,* and five families were resistant to both *H. teres* and *E. graminis.* The value of later generations of composite cross populations as sources of resistance for breeding against pathogens thus seems to have been increased by recombination and natural selection. The appearance of multiply resistant types suggests that there has been no hindrance due to negative correlations. To get an idea of the relationships among resistance to the various diseases, we calculated correlations, as discussed below.

In using multiply resistant lines in plant-breeding programs, it is useful to know whether resistance to one disease (or race) is independent of or correlated with

Table 3. Multiply resistant phenotypes observed within families from the CC II generations studied; the six symbols in each phenotype code, in order, reactions to *H. teres, E. graminis,* and races 40, 61, 72, and 74 of *R. secalis* (R = resistant, S = susceptible); only phenotypes having three or more resistant reactions are listed

Parents	F_8	F_{13}	F_{23}	F_{45}
SSRRSR	SSRRSR	SSRRSR	SSRRSR	SSRRSR
SRRSS	–	–	–	–
–	SRSRRS	–	–	–
–	–	–	RSSRRS	–
–	–	–	–	RSRSSR
			RSRRSR	RSRRSR
–	–	–	–	SRRRSR
–	–	–	–	RRRSSR
–	–	–	–	RRRRSR

Table 4. Coefficients of pairwise correlations between resistance to *H. teres, E. graminis,* and four races of *R. secalis*[a] in parents and generations of CC II

		E. graminis	*R. secalis* races			
			40	61	72	74
H. teres	P	0.035	0.035	-0.190	-0.078	-0.141
	F_8	0.134	-0.149	-0.149	-0.119	-0.057
	F_{13}	0.317*	-0.098	-0.060	-0.042	-0.072
	F_{23}	-0.151	0.505*	0.561*	0.053	0.411*
	F_{45}	0.078	0.301*	-0.033	-0.206	0.211
E. graminis	P		0.151	-0.016	-0.100	-0.180
	F_8		0.003	0.003	0.091	-0.073
	F_{13}		-0.199	-0.096	-0.096	0.100
	F_{23}		-0.114	-0.141	-0.114	-0.133
	F_{45}		0.076	0.159	0.187	0.177
R. secalis Race 40	P			0.665*	-0.100	0.381*
	F_8			0.310*	-0.103	0.666*
	F_{13}			0.848*	-0.044	0.721*
	F_{23}			0.591*	-0.121	0.712*
	F_{45}			0.361*	-0.590*	0.747*
R. secalis Race 61	P				0.412*	0.441*
	F_8				0.694*	0.567*
	F_{13}				0.029	0.860*
	F_{23}				0.216	0.638*
	F_{45}				-0.128	0.364*
R. secalis Race 72	P					-0.066
	F_8					-0.042
	F_{13}					-0.034
	F_{23}					-0.042
	F_{45}					-0.518*

[a] Families segregating for resistance to *R. secalis* were considered as resistant in calculating correlations between *H. teres, E. graminis,* and *R. secalis*

* Significant at 0.05 probability level

resistance to others (and also-ideally-whether observed correlations are due to a common genetic basis, linkage, or epistatic selection). Bird (1982), in a study of cotton, reported significant positive associations between blight resistance and resistance to verticillium wilt and phymatotricum root rot and also between blight resistance and seed-coat resistance to mold. The present study was designed to provide information useful in plant breeding by revealing whether observed resistances are correlated or independent. For this, parents and families were given values 1, 2, or 3 to indicate whether they were susceptible, resistant, or segregating. Using these values, we calculated Pearson product-moment correlation coefficients between resistance to pairs of pathogens for parents and for each of the four generations. The results, which must be regarded as preliminary because sample sizes were small, are shown in Table 4.

The results for pairs of races of *R. secalis* are consistent with those of Muona et al. (1982): Correlations between resistances to races 40, 61, and 74 were positive and significant whereas resistance to race 72 was independent of resistance to the other races except in generation 45. Parental lines did not show any significant pairwise correlations of resistance to *R. secalis, E. graminis,* and *H. teres* (except for those between the races of *R. secalis*). There was no significant correlation between resistance to *E. graminis* and to any of four *R. secalis* races in any generation. In the F_{23} there were significant positive correlations between resistance to *H. teres* and to races 40, 61, and 74 of *R. secalis.* Correlation between resistance to *H. teres* and race 61 of *R. secalis* was not significant in the F_{45}, but correlations between *H. teres* and races 40 and 74 of *R. secalis* were significant at the 0.02 and 0.07 probability levels. The results are thus encouraging in the sense that no negative correlations were found except for those between race 72 and races 40 and 74 of *R. secalis.*

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