Values of Stress Resistance Genes Relative to Dry Weight Accumulation in Wheat Seedlings

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ABSTRACT

Biotic stresses on winter wheat (*Triticum aestivum* L.) seedlings can be extremely damaging. Many genes for resistance to insects and pathogens have been introduced into wheat; however, no quantitative estimates are available regarding the effectiveness of such genes with regard to seedling traits. In this study we used closely related lines derived from backcrossing using a single recurrent parent to assist in estimating genotypic values for four resistance genes: *Pm17*, for resistance to powdery mildew; *Gb2*, for resistance to biotype 'C' greenbug; and two sources of resistance to biotype 'E' greenbug, *Gb3* and *Gb6*. All genes were present in the TAM-105 background. The genotypes were evaluated for dry matter accumulation during a 5-week period, beginning at the two-leaf stage. For each greenbug resistance gene, two initial infestation rates were examined, 0.5 and 5.0 aphids per plant. Powdery mildew damage to susceptible seedlings developed more slowly than did greenbug damage at either infestation rate. Resistance conferred by *Pm17* was completely effective in maintaining seedling dry weight in inoculated vs. uninoculated plants, while greenbug infestation of any of the resistant genotypes at 5.0 aphids per plant resulted in significantly reduced dry matter accumulation, compared to uninfested control plants by the end of the study. In TAM-105, which is susceptible to both greenbug biotypes, reduced dry weight occurred earlier when infested with biotype 'E' than with biotype 'C'. Also, while both TAM-105 and TAM-107 are susceptible to biotype 'E', when infested with that biotype, TAM-105 exhibited reduced dry weight sooner than did TAM-107, which possesses biotype 'C' resistance. These results suggest that biotype 'E' is the more damaging of the two biotypes, but that biotype 'C' resistance confers some delay in development of symptoms to biotype 'E'.

KEYWORDS: gene value, greenbug, powdery mildew

The effects of many stress agents on plants are most severe when stress occurs at early growth stages. This is particularly true for biotic stresses such as insect infestation or disease (Kieckhefer and Kantack, 1988; Duczek, 1989; Kieckhefer and Gellner, 1992; Verma et al., 1976). Slow rates of growth or partial stand loss resulting from these stresses limit the ability of the crop to recover later, even if

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natural or artificial controls are subsequently imposed. In the case of winter wheat, such stresses can be very damaging economically, due to its lengthy vegetative growth period and the common farming practice of using that vegetation for winter pasture for grazing by ruminants. As the economic value of winter grazing can be greater than that of the wheat grain (Shipley and Regier, 1972), decreased rates of seedling dry matter accumulation can be particularly costly.

Backcross breeding programs aimed at introgressing stress resistance genes into agronomically suitable genomic backgrounds have resulted in the development of closely related genotypes differing principally by their resistance genes (Porter et al., 1987; Tuleen et al., 1992). The value of such resistance genes would be expected to vary depending upon environment. The availability of closely related lines differing for specific resistance genes, in combination with controlled environments, thus provides an opportunity to measure gene values. This study was undertaken to evaluate the variation in seedling dry matter accumulation among closely related wheat lines specifically attributable to resistance genes.

MATERIALS AND METHODS

Two cultivars, one germplasm line and a breeding line of winter wheat (*Triticum aestivum* L. em. Thell) were used in this study. One of the cultivars, 'TAM-107', and the germplasm line, 'TX85C5820-5', were backcross products of the second cultivar, 'TAM-105'. TAM-107 carries biotype 'C' greenbug (*Schizaphis graminum*{Rondani}) resistance (*Gb2*) and powdery mildew (*Erysiphe graminis*{DC.f. *tritici* Em. Marchal}) resistance (*Pm17*) derived from rye (*Secale cereale* L.) cv. 'Insave', both present on a 1A:1R chromosome arm translocation, inherited through the wheat cultivar, 'Amigo' (Sebesta and Wood, 1978). TX85C5820-5 carries resistance (*Gb6*) to both biotype 'C' and biotype 'E' greenbugs, but is susceptible to powdery mildew, even though it contains the identical chromosome arm translocation, also from 'Insave' rye (Tuleen, et al., 1992). TAM-105 is susceptible to both greenbug biotypes as well as to powdery mildew. The breeding line used, TXGH12588-105, has pedigree (TAM-105 *4/Amigo)*5//'Largo', and it possesses powdery mildew and biotype 'C' greenbug resistances present on the translocated chromosome arm, as well as biotype 'E' resistance (*Gb3*) inherited from Largo (Tyler et al., 1987).

Plants were grown from seed in vermiculite in 8x8x8 cm plastic pots. Fifteen seeds were germinated in each pot. Populations were thinned to ten plants per pot prior to initiating treatments. Mixed fluorescent and incandescent lighting was provided (about 300 $\mu Em^{-2}sec^{-1}$ at pot level) in a growth chamber, using a 12 h photoperiod. At the two-leaf stage, plants were either infested with biotype 'C' or biotype 'E' greenbugs (at a rate of either 0.5 or 5.0 apterous adults per plant) or inoculated with powdery mildew, by dusting with conidia from previously infected plants. An equal number of uninfested and uninoculated plants were grown as the control group. On the day of inoculation/infestation, and on every third day following, for a period of 36 days, 3 replicate pots were harvested from each treatment group. Plants were gently washed with water after harvest, to remove aphids or mycelia. Total above-ground plant material from each pot was oven dried, and then weighed to the nearest mg.

The genetic value of each resistance gene was estimated by subtracting the

difference between treated seedling dry weights and the untreated seedling dry weights in the resistant genotype from the same difference in the susceptible genotype for any individual time interval. In each case, the most appropriate comparisons are between the most closely related genotypes so that epistatic effects are minimized. In the case of the biotype 'E' resistance derived from Largo (*Gb3*), that comparison would be between the resistant breeding line, TXGH12588-105, and the susceptible variety, TAM-107. For the biotype 'E' resistance derived from Insave rye (*Gb6*), the appropriate comparison is between TX85C5820-5 and TAM-105. For biotype 'C' resistance (*Gb2*) and for powdery mildew resistance (*Pm17*), both derived from Amigo, the appropriate comparison is between TAM-107 and TAM-105.

The experiment was analyzed as a factorial design, with 4 genotypes x 6 treatments x 13 harvest dates and 3 replications. Dry weight data were log transformed prior to analysis of variance, and mean separation, when appropriate, was accomplished by Waller's variation of Duncan's multiple range test or by least significant difference (LSD).

RESULTS AND DISCUSSION

Seedling dry weights over the duration of the experiment are summarized in Table 1, from which several important results can be derived. 1) Over the first 9 days of the experiment, no genotypes or treatments differed significantly ($P=0.05$) from each other. 2) Highly significant ($P<0.01$) differences were found among the genotypes, and among the treatments, after 9 days of treatment. 3) Most significant variation among treatments was attributable to the response of known susceptible genotypes. 4) Significant differences were observed between the treated and untreated plants on greenbug-resistant genotypes exposed to the biotypes for which they express resistance, at the higher infestation rate, near the end of the study. 5) In TAM-105, which is susceptible to all the stress agents examined in the study, the effects of biotype 'E' greenbug were observed sooner and were significantly more damaging in dry matter accumulation than were those resulting from biotype 'C' infestation. 6) Both greenbug treatments were significantly more damaging to dry matter accumulation by TAM-105 than was powdery mildew inoculation. 7) Dry matter accumulation in TAM-105 infested with biotype 'E' greenbug departed significantly from the untreated control sooner than did similarly treated TAM-107, though both of those genotypes are biotype 'E' susceptible.

The relationship among genotypes with respect to dry matter accumulation was largely as expected, based upon known resistance or susceptibility. That is, susceptible genotypes showed evidence of reduced dry weight gain sooner, and more dramatically than did resistant genotypes. This was true for all stress agents examined. The results also point out that resistance is not absolute, at least not for greenbug resistance, in that if resistant seedlings were exposed to enough aphids, under conditions favorable for greenbug development and reproduction, those plants eventually succumbed. This result may also apply to loss of dry weight in resistant lines exposed to powdery mildew, at times longer than were examined here, although in this study we observed no visible symptoms of powdery mildew in

Table 1. Seedling dry weights of four related wheat genotypes exposed to greenbug or powdery mildew treatments.

Days After Treatment	Treatment†	Genotype				F test‡
		TAM105	TAM107	TX85C5820-5	TXGH12588-105	
0	Control	0.21§	0.19	0.25	0.22	ns
	Mildew	0.20	0.18	0.23	0.20	ns
	C 0.5	0.25	0.20	0.22	0.25	ns
	C 5.0	0.23	0.17	0.24	0.21	ns
	E 0.5	0.25	0.18	0.26	0.22	ns
	E 0.5	0.21	0.20	0.22	0.19	ns
3	Control	0.22	0.21	0.27	0.26	ns
	Mildew	0.22	0.22	0.28	0.27	ns
	C 0.5	0.25	0.21	0.30	0.25	ns
	C 5.0	0.21	0.22	0.27	0.28	ns
	E 0.5	0.24	0.20	0.29	0.29	ns
	E 5.0	0.26	0.23	0.26	0.28	ns
6	Control	0.28	0.25	0.29	0.32	ns
	Mildew	0.30	0.26	0.31	0.30	ns
	C 0.5	0.30	0.27	0.30	0.29	ns
	C 5.0	0.33	0.28	0.32	0.33	ns
	E 0.5	0.36	0.28	0.33	0.33	ns
	E 5.0	0.32	0.29	0.30	0.34	ns
9	Control	0.36	0.33	0.33	0.36	ns
	Mildew	0.32	0.30	0.34	0.32	ns
	C 0.5	0.35	0.35	0.36	0.38	ns
	C 5.0	0.32	0.31	0.35	0.39	ns
	E 0.5	0.35	0.34	0.35	0.40	ns
	E 5.0	0.28	0.32	0.34	0.35	ns
12	Control	0.42	0.41	0.39	0.39	ns
	Mildew	0.44	0.36	0.40	0.40	ns
	C 0.5	0.41	0.39	0.40	0.42	ns
	C 5.0	0.28	0.37	0.38	0.41	*
	E 0.5	0.37	0.38	0.41	0.42	ns
	E 5.0	0.18	0.33	0.39	0.39	*
15	Control	0.47	0.49	0.44	0.45	ns
	Mildew	0.51	0.42	0.42	0.44	ns
	C 0.5	0.42	0.44	0.45	0.41	ns
	C 5.0	0.22	0.43	0.43	0.47	**
	E 0.5	0.26	0.37	0.42	0.44	*
	E 5.0	0.10	0.21	0.44	0.45	**
18	Control	0.52	0.54	0.46	0.51	ns
	Mildew	0.50	0.48	0.48	0.49	ns
	C 0.5	0.40	0.51	0.48	0.47	*
	C 5.0	0.17	0.49	0.49	0.50	**
	E 0.5	0.20	0.29	0.47	0.46	**
	E 5.0	-¶	0.08	0.48	0.48	**

Table 1. (continued)

Days After Treatment	Treatment†	Genotype				F test‡
		TAM105	TAM107	TX85C5820-5	TXGH12588-105	
21	Control	0.53	0.57	0.53	0.56	ns
	Mildew	0.51	0.55	0.44	0.56	ns
	C 0.5	0.36	0.55	0.52	0.58	*
	C 5.0	0.09	0.54	0.50	0.54	**
	E 0.5	0.14	0.24	0.51	0.51	**
	E 5.0	-	-	0.51	0.47	ns
24	Control	0.60	0.62	0.57	0.61	ns
	Mildew	0.44	0.59	0.50	0.63	*
	C 0.5	0.20	0.60	0.58	0.57	**
	C 5.0	-	0.55	0.55	0.55	ns
	E 0.5	0.09	0.20	0.56	0.62	**
	E 5.0	-	-	0.57	0.60	ns
27	Control	0.62	0.65	0.63	0.64	ns
	Mildew	0.40	0.64	0.36	0.66	**
	C 0.5	0.17	0.58	0.60	0.62	**
	C 5.0	-	0.62	0.61	0.61	ns
	E 0.5	0.03	0.11	0.64	0.66	**
	E 5.0	-	-	0.61	0.62	ns
30	Control	0.64	0.63	0.66	0.73	ns
	Mildew	0.38	0.68	0.41	0.69	**
	C 0.5	0.04	0.62	0.64	0.68	**
	C 5.0	-	0.59	0.68	0.60	ns
	E 0.5	-	0.05	0.67	0.69	**
	E 5.0	-	-	0.64	0.58	ns
33	Control	0.70	0.68	0.69	0.79	ns
	Mildew	0.29	0.72	0.25	0.74	**
	C 0.5	-	0.66	0.70	0.76	ns
	C 5.0	-	0.57	0.66	0.65	ns
	E 0.5	-	-	0.70	0.71	ns
	E 5.0	-	-	0.62	0.65	ns
36	Control	0.76	0.71	0.81	0.84	ns
	Mildew	0.22	0.73	0.17	0.79	**
	C 0.5	-	0.72	0.75	0.80	ns
	C 5.0	-	0.61	0.73	0.68	ns
	E 0.5	-	-	0.77	0.78	ns
	E 5.0	-	-	0.64	0.64	ns
LSD (P=0.05)		0.16	0.11	0.14	0.18	

†Greenbug treaments were one of the two biotypes, C and E, each applied at two rates, 0.5 and 5.0 aphids per plant, in pots (replicates) containing ten seedlings each.
‡Analysis of variance conducted within each treatment and date of harvest (among genotypes): ns = nonsignificant; *, ** = significant (P=0.5, 0.01).
§Data are means of three replicates (ten plants per replicate).
¶All plants died; these entries were not included in subsequent data analyses.

resistant lines. We also observed differences among the susceptible lines in time of onset of symptoms, and rate of dry weight loss. This was particularly noticeable for the comparison of TAM-105 and TAM-107 exposed to biotype 'E' greenbug. While plants of both lines were susceptible, and eventually died, both symptoms and reduced dry weight gain occurred earlier in TAM-105 than in TAM-107. Possibly, the biotype 'C' resistance in TAM-107 is mildly inhibitory to biotype 'E' greenbugs.

Calculation of the values of specific resistance genes (Table 2) is permitted by the close relationship among these lines, so that neither epistasis nor genotype x environment interaction is likely to confound interpretation of the results. These calculations permit evaluation of the relative effectiveness of each resistance gene to the appropriate stress agent, at least within the genetic background provided by TAM-105. It could be viewed that the least effective resistance was that provided by *Pm17* since the difference between plants carrying resistant vs. susceptible alleles at this locus was less than that for the other loci examined. This is reflected by lower estimates of genetic value for this gene than for the three greenbug resistance loci. On the other hand, during the time period evaluated in the current study, *Pm17* provided absolute immunity to the pathogen, a result not observed for greenbug infestation of any of the sources of greenbug resistance. These results likely relate to the longer period of time required for occurrence of mildew damage than for greenbug damage, so in that sense the comparison may be misleading. Still, the results show that under conditions optimal for the development of either stress agent on wheat seedlings, the greenbug resistances are more valuable. Estimates of genetic value did not differ greatly between the two biotype 'E' greenbug resistance sources, when exposed to similar inoculation levels, although significant differences between control plant dry weight and heavily infested TXGH12588-105 did occur six days earlier than similar significant differences in TX85C5820-5. Estimates of genetic value for the biotype 'C' resistance conferred by *Gb2* were generally smaller than those for either of the biotype 'E' resistance genes, indicating that, at least in the TAM-105 background, any biotype 'E' resistance may be more beneficial than biotype 'C' resistance.

The results of this study suggest that greater attention should be paid to economic threshold values for seedling wheat. This study was conducted under controlled conditions which were near optimal for development of the insects or mildew. Such conditions can prevail in winter wheat fields planted early to permit winter grazing. The higher rate of initial greenbug infestation used in this study was approximately equal to that currently recommended for spray treatment (Boring and Patrick, 1994), while the lower infestation rate was 4- to 10-fold less than the recommended treatment level for seedling wheat. Both levels produced significant reductions in seedling dry weight gain within 2 weeks on susceptible wheat, however. Such dry weight reductions are the observable result of very early structural damage (Morgham et al., 1994), so that even if aphids are removed at the recommended time, the damage may persist, as suggested by measurements of root length and dry weight (Burton and Burd, 1993). Because most wheat in the south central US is grazed by livestock, it is also important in these areas to consider the potential for damage to forage quality in determining spraying recommendations. Perhaps the value of resistance genes to producers may be enhanced by understanding differences in forage quality under seedling infestation.

Table 2. Estimates of genetic value for resistance to greenbug and powdery mildew in wheat seedling dry weight accumulation.

Resistance Gene	Treatment Level†	Days after Treatment												
		0	3	6	9	12	15	18	21	24	27	30	33	36
Gb2	High	NS‡	NS	NS	NS	.121KL§	.208J	.344G	.419EF	---¶	---	---	---	---
	Low	NS	NS	NS	NS	NS	NS	.107L	.153K	.320G	.406F	.595B	.651A	---
Gb3	High	NS	NS	NS	NS	NS	.299HI	.415EF	---	---	---	---	---	---
	Low	NS	NS	NS	NS	NS	.137KL	.287I	.332GH	.486D	.604B	---	---	---
Gb6	High	NS	NS	NS	NS	NS	.288I	.470D	---	---	---	---	---	---
	Low	NS	NS	NS	NS	NS	.129KL	.296HI	.353G	.465D	.577B	---	---	---
Pm17	-	NS	NS	NS	NS	NS	NS	NS	NS	.151K	.224J	.330GH	.448DE	.533C

†Greenbug infestation rates were 0.5 aphids per plant (low) or 5.0 aphids per plant (high).
‡NS = non significant ($P > .05$).
§Means followed by the same capital letter are not significantly different by Waller-Duncan test (K ratio = 100).
¶All susceptible plants died.

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