INHERITANCE OF RESISTANCE TO ALTERNARIA BLIGHT IN SUNFLOWER RESTORER LINES

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Abstract

Little is known about the inheritance of Alternaria blight resistance in sunflower. In particular, the expression of resistance from individual inbred lines in hybrid combinations is not well documented. In this study, two resistant R-lines (male lines) were selected for evaluation in hybrid combination with six A-lines (female lines). Five proprietary A-lines and the public line HA 89 were crossed with the two restorer lines, to produce twelve F1 hybrids. These hybrids, their parental lines and three commercial hybrids were exposed to generated epidemics of *A. helianthi* at two field sites. An area under the disease progress curves (audpcs) was calculated for each line, from disease severity ratings (DSRs) taken from budding to late flowering. The commercial A-lines were as susceptible to *A. helianthi* as the susceptible line A89. The two restorer lines had high levels of resistance. In general, resistance of the F1 hybrids was greater than their midparent estimates, indicating that the two restorer lines exhibited a high degree of dominance for genes controlling resistance.

Introduction

Carson (1985a) noted that Alternaria blight of sunflower caused by the fungus *Alternaria helianthi* (Hansf.) Tub. and Nish., is a potentially devastating disease. His comment refers to the lack of resistance to blight in the sunflower gene pool, and the rapid growth and short reproductive cycle of the pathogen under favourable conditions. Warm, moist conditions favour infection processes, but extended periods of leaf wetness are required to elevate disease to epidemic levels. Such conditions occur in tropical and subtropical regions where monsoonal activity accelerates disease progress throughout the growing period. Sunflower plants are most susceptible to the disease at flowering, when disease levels are likely to be greatest. High inoculum loads and wet conditions can result in rapid defoliation of plants at this stage. Poor grain fill and/or seed set occur, depending on the timing and severity of the epidemic.

Resistance to *A. helianthi* is of a quantitative nature (Mehdi et al., 1984; Carson, 1985; Morris et al., 1983; Lipps and Herr, 1986; Kong et al. 1997) and hence is difficult to detect (Kong et al. 1995) and develop in a breeding program. This is because quantitative resistance is conditioned by the collective action of many genes of minor effect that either add to or subtract from the expression of resistance (Mather and Jinks, 1977). Moreover, recognition of the resistant phenotype is confounded by the influence of environment on the expression of resistance (Falconer, 1989).

For a hybrid crop such as sunflower, this implies that the genes contributed from both the male and the female parents are collectively responsible for the level of resistance that can be expressed. Consequently, male and female lines that independently show high

levels of resistance may not do so in combination, or alternatively, they may produce hybrids of equal or greater resistance than either parent, depending on gene action.

Generally, sunflower breeders would select for disease resistance in male restorer lines (R-lines). They may or may not subsequently carry the resistance during conversion to a female line, depending on the degree of difficulty and the prevalence of disease at the time of selection. Any characterised levels of resistance to Alternaria blight therefore tend to be carried in male parents and testcrossing of males to females must be conducted in order to determine the level of resistance in the resulting hybrid.

Two restorer lines with moderate to high levels of resistance to Alternaria blight were evaluated in hybrid combination with six commercial female lines (A-lines) obtained from local seed companies. Epidemics of Alternaria blight were generated at two field sites and measurements of disease severity were used to evaluate the levels of resistance arising from the combinations.

Materials and Methods

Hybrid Production and Field Trial Design. Two restorer lines, code 10020.11.4.20 and 10008.3.3.5 were selected on the basis of blight resistance and agronomic qualities (Kong, 1997) and for this experiment were designated R1 and R2 respectively. R1 and R2 were crossed with the public female line HA 89 as well as 5 proprietary A-lines, to produce twelve F1 hybrids. The A-lines, hybrids and their experiment codes are shown in Table 1.

Table 1. *Hybrids that were produced from crosses between six A-lines and two selected R-lines*

Codes for A-lines	Source of A-lines	Codes for hybrids made with R1	Codes for hybrids made with R2
A1	HA 89 is a public line	H11	H21
A2	Pacific Seeds Pty Ltd	H12	H22
A3	Pacific Seeds Pty Ltd	H13	H23
A4	Agseed-Research Pty Ltd	H14	H24
A5	Agseed-Research Pty Ltd	H15	H25
A6	Pioneer Hi-Bred Pty Ltd	H16	H26

R1= Refers to the restorer line 10020.11.4.20; R2= Refers to the restorer line 10008.3.3.5; H = Refers to the A- and R-line combinations used to produce each F1 hybrid.

Seed of the designated crosses was produced in the field in 1993 inside open-weave fabric tents designed to exclude insects. A beehive containing a nucleus colony of bees was placed inside each tent at flowering to facilitate cross-pollination. Seed was harvested from each A-line in January, 1994.

The two R-lines, six A-lines, twelve hybrids and three commercial hybrids, Hysun 45CQ (Pacific Seeds), Suncross 41 (Agseeds Research) and Advantage (Pioneer Hi-Bred), were planted at two field sites on 10 February, 1994. Site 1 was at the Queensland Department of Primary Industries (QDPI) Research Station located at Gatton and Site2 was at QDPI research station at Kingsthorpe. These sites are 70 km apart and the Kingsthorpe site is 500 m higher in elevation.

Seed was planted in 12.5m rows, with an inter-plant spacing of 0.2m and an interrow spacing of 0.75m. Lines were randomised in three replications; however the A-lines and R-lines were kept together as a randomised group within each replicate (block) to reduce the adverse effects of competition exerted by the more vigorous hybrids. Two rows of the

susceptible line B89 were planted perpendicular to the ends of each treatment row and extended the full width of each replicate. These rows were spray inoculated with spores of *A. helianthi* when plants were at the V4 growth stage (Schneiter and Miller, 1981), then again 1 and 2 weeks later. Overhead misting was applied for 8 h following each inoculation and then daily for 3–4 days after the inoculation, for a period of 4–6 h. Disease assessment began at Site1, 61 days after planting when plants were at growth stage R2–R4 and at Site2, 67 days after planting when plants were at growth stage R1–R3. Plants at Site1 were assessed again at 70, 78, 85 and 92 days after planting. Plants at Site 2 were assessed again at 78, 84 and 91 days after planting. Ten plants at intervals of about 1m were assessed in each row, allowing a space of about 1.75 m from the ends of the rows to the first and last plants assessed. The 3rd or 4th pair of leaves of each assessed plant was marked with red paint, so that the same leaves were assessed at each assessment time. The proportion of diseased leaf tissue was determined for each marked leaf pair using a modified version of the pictorial key developed by Allen et al. 1983.

Analysis of Data. Area under the disease progress curves (audpcs) were calculated for all plants assessed at both sites. Data were log-transformed before comparing audpc means with Scheffe's test for significant differences (SSD). The difference between the midparent audpc and the F1 hybrid audpcs was calculated for each sunflower line. Linear regression and Spearman's rank correlation were used to determine the degree of correlation between the resistance of sunflower lines grown at each field site. A combined analysis of variance was conducted and variance components partitioned to provide estimates of genotype x environment interactions. The g x e interaction was estimated as $\sigma 2ge$ = (interaction mean square - error mean square).

Results

Audpcs of Parental Lines and F1 Hybrids. Disease intensity (audpc) at Site 2 was greater than that observed at Site 1 (untransformed data, Tables 2 and 4). The restorer lines R1 and R2 were the most resistant parental lines at both sites (Table 2). Overall, there was no difference in resistance between the A-lines; however at Site 1, A4 had a significantly lower level of infection than all other A-lines.

Table 2. Area under the disease progress curves (audpcs) for the R-and A-lines grown at field sites 1 and 2. Values followed by the same letter are not significantly different.

	Audpc means			
	Site 1		Site 2	
Parent lines	transformed	untransformed	transformed	untransformed
R1	4.148 b	68.38	4.143 c	74.53
R2	4.265 b	82.90	4.180 c	87.87
A1	4.915 a	165.23	4.954 a	186.63
A2	4.851 a	137.57	5.228 a	233.77
A3	4.762 a	134.83	4.953 ab	180.95
A4	4.328 b	83.69	4.675 b	130.68
A5	4.977 a	155.49	4.934 ab	173.65
A6	4.760 a	137.48	4.656 b	147.68

Both transformed and untransformed data are presented. Untransformed data were used to generate the midparent values for the F1 hybrids shown in Table 4. Audpc means were compared using log-transformed data.

At Site 2, H23 was the most resistant hybrid, but there was no significant difference in resistance between any of the other hybrids (Table 3). At Site 1, H21 was the most susceptible hybrid and H25 the most resistant. All other hybrids had the same level of resistance.

Audpcs for the sunflower lines grown at Sites 1 and 2 were well correlated. Spearman's ranking of the lines gave a correlation coefficient of 0.750, while a coefficient of determination (R) of 0.895 was obtained from the regression of audpcs at Site1 with Site2.

Table 3. Area under the disease progress curves (audpcs) for F1 hybrids grown at field sites 1 and 2. Values followed by the same letter are not significantly different.

	Audpc means	
Hybrid lines	Site 1	Site 2
H11	3.765 ab	4.074 a
H12	4.087 a	4.169 a
H13	3.830 ab	4.255 a
H14	3.902 ab	4.212 a
H15	4.019 ab	4.417 a
H16	4.009 ab	4.281 a
H21	3.458 b	3.880 a
H22	3.763 ab	4.258 a
H23	3.572 ab	3.481 b
H24	3.727 ab	4.088 a
H25	2.781 c	3.883 a
H26	3.791 ab	4.220 a
Hysun 45CQ	3.489 b	3.786 a
Suncross 41	4.002 ab	4.111 a
Advantage	3.977 ab	4.444 a

Audpc data were log-transformed for analysis. Transformed data are presented.

Differences Between F1 Hybrids and Midparent Audpcs. Midparent audpcs for each hybrid (Table 4) were calculated by averaging the untransformed audpcs for the A- and R-line parents shown in Table 2. Overall, the hybrids were more resistant than was expected from their midparent values. This is reflected in the negative values that were obtained when the midparent audpcs were subtracted from the F1 hybrid audpcs (F1-MP; Table 4). At both field sites, all hybrids based on R2, except for H24, were significantly more resistant than expected from the calculated midparent values. At Site 1, five of the six hybrids based on R1, were significantly more resistant than expected from the calculated midparent values, but at Site 2, only three of these had audpcs smaller than their midparent. The hybrid H14 had the same resistance (not significant) as the midparent at both field sites. At both sites, hybrids made with line R2 were on average slightly more resistant than hybrids made with R1.

Table 4. Differences between F1 hybrid and midparent audpcs calculated from untransformed area under the disease progress curves (audpcs) for plants grown at sites 1 and 2.

	Audpc means					
	Site 1			Site 2		
Hybrid code	F1 hybrid	Midparent	F1 - MP	F1 hybrid	Midparent	F1 - MP
H11	46.87	116.76	-69.89 *	80.74	130.06	-49.26 *
H12	65.64	102.98	-37.34 *	81.34	154.15	-73.80 *
H13	50.08	112.46	-62.38 *	106.95	127.74	-20.79
H14	53.48	76.00	-22.52	91.13	102.61	-11.48
H15	63.28	111.90	-48.62 *	107.16	124.10	-16.94
H16	57.52	102.93	-45.41 *	104.50	111.11	-66.10 *
Mean	**56.15**	**102.03**	**-47.69 ***	**95.57**	**124.96**	**-39.72 ***
H21	40.89	123.60	-82.71 *	68.81	137.25	-60.29 *
H22	63.53	109.80	-46.27 *	100.51	160.80	-60.30 *
H23	46.44	108.47	-62.03 *	60.83	134.40	-75.70 *
H24	52.97	82.90	-29.93	89.64	109.28	-19.64
H25	21.80	118.80	-97.00 *	74.86	130.76	-55.84 *
H26	54.29	110.28	-55.99 *	86.85	117.78	-30.93 *
Mean	**46.65**	**109.50**	**-62.32 ***	**80.25**	**131.71**	**-50.45 ***

* = F1 mean and midparent are significantly different.

Combined Analysis of Variance for Sites 1 and 2. Table 5 shows the combined analysis of variance for the two field sites. As expected, there were significant differences between sunflower lines. Significant differences between replicates within sites indicated that disease was not uniform across the trial sites. The lines x sites interaction was not significant, which suggests that the g x e interaction was small. The estimate of g x e from Table 5 is -0.105, which must be assumed to be zero.

Table 5. Combined analysis of variance for audpcs of sunflower lines grown at the two trial sites.

Source	Df	Mean Square	F
Sites	1	2.241	3.0 *
Reps within sites	4	0.618	3.1 *
Lines	22	1.160	5.8 **
Lines x sites	22	0.095	<1
Error	88	0.199	

** = significant at 0.05 level*
*** = significant at .01 level*

Discussion

In almost all cases, the cross between the resistant restorer line (male line) and the susceptible female parent (A-line) resulted in a hybrid with resistance that was not only greater than the expected mid-parent values but equal to the R-lines themselves. These positive deviations of the F1 from the midparent value indicate a high level of genetic dominance. That is, genes inherited from the resistant restorer have a large effect on resistance, and overall, their effect is augmented rather than diminished through the combination of genes from the susceptible parent. In this study, genes in the susceptible

A-lines appear to have contributed significantly to resistance. Although the data appear to indicate qualitative resistance, F2 analysis of the restorer lines did not produce a discrete distribution for resistance (G. Kong, unpublished data). How much of the observed gain in resistance can be attributed to genes involved specifically in resistance mechanisms is unknown. Improvements in resistance associated with the crosses may also be due to general changes in vigour, and physical attributes that present passive barriers to disease. Whatever the mechanisms, it seems clear that when combined, genes of small effect can produce good levels of resistance to Alternaria blight. Such genes can only be detected through the evaluation of crosses.

The evidence for this high degree of dominance among the gene(s) contributing to resistance was found for all crosses except for those involving the line A4. At Site 1, A4 was the only A-line to exhibit resistance equal to the resistant R-lines and at Site 2 it also had the greatest resistance of the A-lines. It is therefore unexpected that crosses with A4 should have the least amount of gain in resistance. The additive-dominance model (Mather and Jinks, 1977) is often used to explain gene action and in the case of A4, the additive effects appear to be as expected but the dominance effects expected from the R-lines have been neutralised. In order to determine the degree of dominance (non-additive) relative to additive effects, a structured mating design such as the diallel would be required.

The lines used in this study also showed no interaction with environment for expression of resistance phenotype. Sensitivity to environment is often a feature of quantitative traits; however, the genes involved in resistance in these lines behave more like genes that confer qualitative traits. The high correlation coefficient for the ranking of lines across the two test sites also confirms this insensitivity to environment.

When this study began, there weren't any A-lines publicly available that had resistance to *A. helianthi.* In general, the proprietary A-lines used in this study were susceptible to *A. helianthi* and had levels of resistance similar to the susceptible standard line, HA 89. Because of the way that sunflower hybrids are produced and the nature of resistance to *A. helianthi,* it is likely that levels of resistance in hybrids would be greatly improved if resistance could be incorporated into both parents. From a breeding point of view, this is a difficult and time consuming task, as resistance would need to be maintained throughout the process of converting a superior inbred line to an A-line. If gains in resistance following a single cross were sustainable with increasingly resistant parents, then theoretically, highly resistant hybrids could be produced. The results of this study are encouraging in this regard, as five of the six females parents, whilst susceptible to Alternaria blight, produced hybrids with greater resistance than expected.

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