NEW CONSIDERATIONS FOR WHITE ROT GENETIC RESISTANCE

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Abstract

Different characters related to sunflower white rot disease are evaluated as possible components of partial resistance. The feasibility of detecting genotypic effects across environments and obtaining hybrids with low disease severity after crossing selected inbred lines is explored. A series of 20 hybrids obtained in a factorial genetic mating design were infected with *Sclerotinia sclerotiorum* on capitula in two environments. Disease severity was estimated 27 and 40 days after infection (DAI). Mean values for severity were higher at the second evaluation date. Highly significant effects for hybrids were detected. The absence of hybrid-environment interaction suggested similar hybrid performance across locations. The lack of significance of the coefficient of rank correlation indicated some crossed inversions when hybrid rankings at 27 and 40 DAI were compared. The degree of genetic determination indicated that the major portion of the total variation was due to non-genetic differences among hybrids. The general combining ability effects for males and females were significant; however, those of males were most important because of the higher variability and significance of their effects. The specific combining ability was significant at 27 DAI. Some inbred lines demonstrated being good combiners to produce hybrids with low disease severity. The use of this character in breeding for white rot resistance is discussed.

Introduction

Sunflower (*Helianthus annuus* L.) is susceptible to *Sclerotinia sclerotiorum* (Lib) de Bary attacks. The pathogen produces capitula white rot, an important disease in many sunflower growing regions (Gulya et al., 1997).

Previous studies (Castaño et al., 1993) have shown that sunflower resistance to white rot is partial and quantitatively inherited. More resistant genotypes show a reduced number of diseased capitula (disease incidence) with a longer incubation period when they are compared with highly susceptible ones. These two characters, which are independently and easily evaluated in the field, are regularly being used to detect genetic diversity in sunflower for *S. sclerotiorum* resistance.

The severity of lesion (SEV) on diseased capitula could be another component of partial resistance that can be incorporated into selection schemes. Thus, selection considering these three components (i.e., disease incidence, incubation period and lesion severity) could allow accumulation of favorable alleles controlling each one of them. If the genes controlling these traits have different effects, the selection process will result in a more complex type of resistance while time to obtain new cultivars with a good level of *S. sclerotiorum* resistance could be reduced.

A research program was started to study the level of difficulty of measuring SEV in the field, as well as to detect genetic variability of responses and to conduct inheritance studies. In a previous work (Castaño et al., 2001) genotypic differences for SEV were demonstrated using a low cost, simple and relatively quick method. In the present study, we wanted to know if these genotypic performances were maintained across environments and if it was possible to obtain new hybrids with low disease severities by crossing selected inbred lines.

Materials and Methods

Twenty single-cross sunflower hybrids, developed using the factorial genetic mating design or North Carolina II, combining four cytoplasmic male sterile lines and five restorer male lines, were used (Table 1). These 9 inbred lines were selected according to Godoy et al. (2000) because they showed, in hybrid combination, variability to *S. sclerotiorum* incidence.

Experiments were conducted in two locations: Balcarce and Camet, where hybrids were sown according to a complete randomized block design with two replications.

Sclerotinia infections were made according to a published protocol (Vear and Tourvieille, 1984). All capitula were inoculated in the R5.3 stage of sunflower development (Schneiter and Miller, 1981) or its homologous E3 (Cetiom, 1992) with approximately 25,000 ascospores. Inoculations were made twice a week, each plant being infected once.

Environments at each location differed mainly in the way that sunflower capitula were treated after inoculation. At Balcarce all capitula were immediately covered with Kraft-type paper bags and a commercial overhead sprinkler irrigation system applied 5 mm of water, twice per week, until the conclusion of the experiment. Conversely, at Camet the infected capitula were left uncovered and an overhead micro-sprinkler system irrigated two or three times a day keeping a high humidity level throughout the day.

In every diseased capitulum, both the area of the inflorescence side and the area showing white rot symptoms were estimated at 27 and 40 DAI using a crossed, flexible and numbered rule (Castaño et al., 2001). Disease severity in % (SEV %) was calculated afterwards as the proportion of capitulum with white rot symptoms.

Analyses of variance were individually made for both locations and the equality of error variances was checked by the Bartlett's test for homogeneity of variance. A nested analysis of variance combined over locations with a two-factor mixed model (fixed genotypes and random environments) was calculated. The hybrid sum of squares was partitioned into variation due to females (F), males (M) and F x M interaction; the main effects of F and M were equivalent to general combining ability (GCA), and the F x M interaction was equivalent to specific combining ability (SCA) effects. GCA effect and SCA effect were calculated according to Beil and Atkins (1967) for each inbred line and hybrid. Standard errors for GCA effect of F and M inbred lines and SCA effect were calculated using the Cox and Frey's (1984) method. Two-tailed t-tests were used to test the significance of the GCA and SCA effects following Singh and Chaudhary (1985). To test the significance of association between the two white rot observation dates, Spearman's nonparametric test was performed. The degree of genetic determination was calculated according to Falconer (1981).

Results and Discussion

Hybrid Performance. Table 1 shows SEV% mean values at 27 and 40 DAI in two environments. General means of 40% and of 90% were observed at each date. At 40 days, the SEV% mean value was 125% higher than the one estimated at 27 days. At 40 DAI a higher level of precision in the experiment was estimated (CV=9%) compared with that of 27 DAI (CV=46%).

Table 1. Mean values of white rot severity (SEV%) estimated at 27 and 40 days (in italics) after *S. sclerotiorum* inoculations on 20 F1 sunflower hybrids at Balcarce and Camet.

Males	R122	R152	R161	R199	PAC1	Mean
Females						
DK1	68	51	31	36	30	43
	96	*99*	*90*	*84*	*94*	*92*
DK3	32	35	26	74	29	39
	89	*90*	*75*	*83*	*90*	*86*
DK4	33	50	20	38	31	34
	96	*90*	*89*	*79*	*84*	*88*
SD	60	37	45	50	27	44
	98	*94*	*99*	*86*	*87*	*93*
Mean	49	43	31	49	29	40
	95	*93*	*88*	*83*	*89*	*90*

LSD (*p=0.05*) values: 27% (27 d) and 12% (40 d); CV= 46% (27 d) and 9% (40 d)

Analysis of variance (Table 2) showed no differences between environmental means. Neither the different management practices followed after infection at each site nor the

differences in other environmental variables that could exist between locations produced significant variation between environmental means for SEV%.

Highly significant (p=0.01) differences were detected among hybrids at 27 and 40 DAI. This indicates that the tested methodology provided a means to separate hybrids according to SEV% performance. Hybrid by environment interaction was not significant; therefore, the relative performance of hybrids was similar across locations at both observation dates, which suggests that the evaluation of SEV% in these hybrids could be done in a single environment.

Table 2. Analyses of variance combined across environments for white rot severity (SEV%) scored at 27 and 40 days after *S. sclerotiorum* inoculations.

Sources of Variation	d f	F (27 d)	F (40 d)
Environments (E)	1	3.95	1.32
R / E	2	2.53	6.36**
Hybrids (H)	19	2.50**	2.62**
Females (F)	3	1.10	3.87*
Males (M)	4	4.43**	5.28**
F x M	12	2.21*	1.42
H x E	19	1.28	1.51
F x E	3	0.17	0.46
M x E	4	2.04	1.80
F x M x E	12	1.31	1.68
Error	38		

**, *: Significant at *p=0.01* and *p=0.05*, respectively

The LSD test was used to separate genotypes in groups of similar levels of resistance according to whether their SEV% values were statistically equal or not to the maximum and minimum values in this experiment. At 27 DAI, 14 hybrids were in the group 1 (G1) of higher resistance level, the other 6 were in the group of lower level of resistance (G2). At 40 days, 6 hybrids were in G1 and 13 hybrids were in G2; the hybrid SD x PAC1 was by itself in G3, a group with an intermediate level of resistance between G1and G2.

Spearman's rank correlation showed no significance with a coefficient of rs= 0.28. Therefore, the ranking of hybrids according to their SEV% values was independent regardless of the observation date. Forty percent of hybrids were in groups of similar levels of resistance at each observation date. Hybrids DK3 x R161, DK4 x PAC1, DK1 x R199, and DK4 x R199 were classified in the G1 group, and DK1 x R122, SD x R122, DK1 x R152 and DK4 x R152, coincided in the G2 group at both observation dates.

Genetic Determination and Combining Ability Studies. The degree of genetic determination varied from DGD=21% to DGD=18% at 27 and 40 DAI, respectively. More than 75% of the SEV% total variation is therefore attributable to non-genetic differences among hybrids. The low values of DGD could be affected by the inbred lines, which were chosen for this experiment because they produced hybrids with a wide range of disease incidence responses, presumable, and independent variable from SEV. Perhaps another set of lines specifically selected for their higher genetic variability in cross combinations for the SEV trait would have to be evaluated to obtain a more favorable DGD estimate.

Table 3 shows the GCA and SCA effects for the inbred lines. Negative values in GCA indicate that mean parental effects are lower than the general mean. Negative values in SCA effects describe those hybrids showing a value of SEV% lower than expected using the GCA

parental effects. Analysis of variance (Table 2) detected highly significant (p=0.01) differences for GCA effects for males at each observation date. For females lines, significant (p=0.05) effects were observed for GCA at 40 DAI and for SCA at 27 DAI. There were no interaction effects between inbred lines and environment. All combining abilities effects, therefore, were similar in both locations.

Table 3. General and specific combining ability effects of 9 sunflower inbred lines used in a North Carolina II mating design to produce hybrids evaluated for white rot severity (SEV%) at 27 and 40 days (in italics) after *S. sclerotiorum* inoculations.

Males	R122	R152	R161	R199	PAC1	GCAf[$]
Females						
DK1	16.7[(#)]	4.8	-2.6	-16.9	-2.1	3.1
	-1.6	*2.9*	*-1.4*	*-2.0*	*2.0*	*2.9*
DK3	-15.4	-7.3	-3.4	25.7	0.3	-1.0
	-1.7	*1.1*	*-9.1*	*4.2*	*5.4*	*-4.1*
DK4	-9.5	12.4	-5.3	-5.4	7.8	-5.8
	3.4	*-1.6*	*2.7*	*-1.9*	*-2.6*	*-1.8*
SD	8.2	-9.9	11.2	-3.5	-6.1	3.6
	-0.1	*-2.4*	*7.8*	*-0.4*	*-4.8*	*3.1*
GCAm[&]	8.4	3.0	-9.6	9.2	-11.1	
	5.3	*3.4*	*-1.1*	*-6.6*	*-0.9*	

[&]Male inbred line GCA effects: SE gcam= 5.9, then GCAm=x±16.4 (27 days), SE gcam= 2.1, then GCAm=x±5.8 (40 days).
[$]Female inbred line GCA effects: SE gcaf= 1.5, the GCAf=x±4.8 (27 days).
[#]Male x Females SCE effects: SE sca= 8.2, then SCA=x±17.9 (27 days).

Estimated GCA effects for males varied from 8% to 11% at 20 DAI and from 5% to 7% at 40 DAI (Table 3). The inbred lines PAC1 and R161 showed favorable effects at both dates; therefore, they can be considered as good combiners in breeding for the production of hybrids with low SEV%. The inbred line R199 showed favorable effects at 40 DAI but non-favorable ones at 27 DAI. This difference could be a consequence of its combination with the inbred line DK3; at 27 DAI, hybrid DK3 x R199 had the highest SEV% value (Table 1), but at 40 DAI its SEV% only increased 12% compared with other hybrids, which increased up to 345% (e.g., DK4 x R161). This factor could have influenced the appearance of contrasting GCA effects in this inbred male line.

The GCA effects for female lines varied from 3.1% to 4.1% at 40 DAI. Lines DK3 and DK4 showed favorable GCA effects and can be positioned as good combiners. The SCA effects varied from 26% to -17% at 27 DAI. The hybrid DK1 x R199 showed the largest FxM effect difference from what was expected.

In general, a good agreement is observed between inbred lines GCA effects for SEV% and those found in a previous disease incidence study (Godoy et al., 2000). Two inbred lines should be highlighted however: SD and DK4. The former showed favorable GCA effects for disease incidence but unfavorable ones for SEV%. Conversely, DK4 showed favorable GCA

effects for SEV% but unfavorable ones for disease incidence. This suggests the need to estimate the GCA effects for both characters in order to choose superior lines to develop hybrids with improved resistance levels to each trait.

The presence of significant additive gene effects for males (at 27 and 40 DAI) and for females (only at the second date) (Table 2) indicate that breeding programs could utilize this gene action to develop inbred lines with low disease severities after *S. sclerotiorum* infection. The non-additive gene effects at 27 DAI should also be taken into consideration.

Conclusions

Although the genetic determination is somehow low, it seems that selection for SEV% could more efficiently be carried out at 40 DAI because a higher control of the data variability was obtained. On this date, it was possible to detect differences among hybrids that had similar performances at 27 DAI (e.g., DK3 x R161 and DK1 x PAC1). At 40 DAI, plants are closer to physiologic maturity and, therefore, there is a shorter period of time left in which diseased capitula could be destroyed before they could be commercially harvested. Presence of both favorable combining abilities and additive gene action would facilitate the process of breeding programs to develop inbred lines and hybrids with a good level of resistance to the SEV% at 40 DAI. However, further studies are necessary to know whether SEV fulfills other requirements (e.g., none or low additive correlation with disease incidence and incubation period) to be established as a new component of the white rot partial resistance system.

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