White rot resistance, seed weight and seed oil content in sunflower test-crosses

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ABSTRACT

In Balcarce, we wanted to obtain sunflower restorer R inbred-lines capable of transmitting to their off-spring a suitable level of partial resistance to white rot, without being inferior for other agronomic attributes. A series of test-crosses obtained by crossing new R inbred-lines with a tester were evaluated. Preliminary results indicated that some R inbred-lines had favourable effects concerning resistance for at least one phase of the white rot development in capitula, as well as seed weight and seed oil content. The level of disease resistance was independent of both agronomic characters, measured on healthy plants. During the cultivar development it should be possible to obtain sunflower hybrids with moderate level of resistance to white rot combined with high seed weight and oil content.

Key Words: biological cost – breeding – disease resistance – *Sclerotinia* – seed oil content – seed weight

INTRODUCTION

White rot symptoms caused by *Sclerotinia sclerotiorum* infections on capitula reduce seed yield and oil content in sunflower cultivars in relation to disease intensity (Gulya et al., 1997). Therefore, it is necessary to develop sunflower hybrids with a suitable level of disease resistance and adapted to be grown in environments where there is a risk of white rot.

In sunflower, white rot disease can be considered as being composed of different phases of a process beginning with the infection during flowering, followed by mycelium invasion in the parenchyma tissues during the grain-filling, and ending with sclerotia formation at maturity. Partial resistance of genotypes can be evaluated by means of indicators related to each of these phases (Castaño, 2007). For example, for the disease incidence and the relative incubation period (Vear and Tourvieille, 1984), the beginning of the disease development (penetration - initial mycelium growth) was measured. With disease severity (Russi et al., 2004) as well as daily white rot progress (Castaño and Giussani, 2006) the severity of intermediate and final phases (mycelium invasion and sclerotia formation) of the white rot development can be estimated

At Balcarce we developed a series of restorer R inbred-lines after inbreeding and selection from old and modern sunflower cultivars. Because this germplasm is potentially interesting for seed weight and oil content, it became necessary to evaluate the capacity of the R inbred-lines to transmit both white rot resistance, and the agronomic characters to their off-spring in order to define which of the lines should usefully be continued in the sunflower breeding program.

In this study, a series of R inbred-lines were evaluated, through their test-cross behaviour, for their reaction to the different phases of white rot development, as well as for the seed weight and seed oil content produced by plants without symptoms.

MATERIALS AND METHODS

Sunflower genotypes

46 test-cross hybrids, with sufficient seed, were produced by pollinating the male-sterile inbred-lines CMS GU and/or CMS GB with 37 restorer R inbred-lines selected from Argentine, French and Italian commercial hybrids, and with the line PSC8 of well known performance to white rot (Castaño et al., 1993). The lines GU (Serre et al., 2004) and GB (F.Vear, pers. com.) were bred by INRA, France, and showed a high level of white rot symptoms quite rapidly after infection in France.

Two sunflower cultivars, PARAÍSO 20 and ACA 884, were used as checks for disease incidence since they showed moderate resistance to this variable in more than 70% of trials carried out in the last decade at Balcarce. In addition, the cultivar VDH 487 was utilized as check for the seed weight and seed oil because of its good performance during the 2005/06 and 2006/07 seasons in the southern sunflower growing area in Argentina (Quillehauquy et al., 2007).

Experiment
The test-crosses and the three sunflower cultivars were sown in the field following a randomized complete block design with two replicates. Plots had at least 15 plants. The lines GU and GB, in the male fertile versions (B), were grown beside this experiment.

Inoculation and disease variables measured
The inoculation protocol of Vear and Tourvieille (1984) was used. The floral surface of 12 plants/plot in the R5.3 sunflower stage (Schneiter and Miller, 1981) (= F3.2, Cetiom, 1992), was sprayed once with an aqueous suspension containing about 25000 ascospores. Inoculated inflorescences were covered with Kraft paper bags until the end of the experiment. Twice weekly irrigations of approximately 5 mm each were made with sprinklers until the sunflower maturity stage.

Capitula were felt twice a week from 15 days after inoculation until first white rot symptoms appeared. Then, each capitulum was examined every 7 days until the end of the experiment. At each observation, the date and the proportion of the diseased capitulum area were scored. The following variables were quantified: 1) disease incidence (%), at the M4 stage, 2) relative incubation period, 3) disease severity, at 40 days after inoculation (40 dai), R8-9 stages (= M2), 4) maximum disease severity reached at the M4 stage and, 5) daily white rot progression (%). An average was calculated per plot for the last four white rot variables.

Seed weight and Seed oil content
The capitula of uninoculated plants (at least three per plot) were covered with netting bags. At maturity, capitula were harvested and seeds weighed. Means per plant and per plot were calculated Seed-oil percentage was determined by nuclear magnetic resonance (NMR) and a mean per plot estimated.

Statistical analyses
Analyses of variance using two criteria of classification (genotypes and blocks) were made, by means of the GLM (SS type III) procedure of SAS. The LSD values were calculated and, in addition, a correlation coefficient between white rot resistance and seed weight and seed oil content obtained. All the analyses were based on Reza-Hoshmand (1998).

RESULTS AND DISCUSSION

Means of each variable measured are shown in Table 1. In 5 of the 7 variables, the coefficient of variability was over 30%. These relatively high values could be related to the insufficient environmental humidity during the experiment despite the irrigations carried out.

White rot variables
General disease incidence reached 23.8% and the range between extreme incidence values was 63.3%. The checks PARAÍSO 20 y ACA 884 had 13.6% and 28.1% of diseased plants, respectively. The disease incidence for the line GU (B) was 40.6% and for GB (B) 34.2% (data not shown). In checks, the disease incidence was 5% (ACA 884) and 12% (PARAÍSO 20) lower than estimated mean values from 10 experiments made previously at Balcarce (Quillehauquy et al., 2007). Concerning the line GU (B), the relative number of diseased capitula was 59.3% lower than the average value reported by Serre et al. (2004) after 13 trials made in France. In relation to the bibliography, the lower mean values of checks as well as in the line GU (B) could be related to inadequate environmental humidity during disease development. Analysis of variance detected significant (α=0.01) differences between genotypes.

Table 1. Average responses of sunflower test-crosses and commercial hybrids to *S .sclerotiorum* inoculations and seed weight and seed oil in plants without white rot symptoms.

VARIABLES[1] ➔	INC (%)	RIP	SEV-40 (%)	SEV-MX (%)	WRP (% / d)	SW (g/cap)	SO (%)
GENOTYPES							
Test-cross							
GB x R1	* 25.0	& 1.38	* 8.3	45.0	1.6	10.0	& 46.8
GB x R2	* 11.8	1.12	* 8.6	* 32.5	1.0	9.4	& 50.6
GB x R3	55.6	0.72	81.7	88.0	4.5	15.0	& 49.7
GB x R4	36.4	& 1.31	* 18.0	44.6	2.2	12.2	& 50.7
GB x R5	* 20.9	1.07	* 51.7	90.0	4.7	11.9	& 49.9
GB x R6	33.1	& 1.18	57.4	88.1	9.2	& 24.5	& 49.7
GB x R7	* 17.4	0.93	87.1	100.0	9.1	14.8	& 50.6
GB x R8	* 0.0	& 1.52	* 0.0	* 0.0	# 0.4	15.6	42.1
GB x R9	* 17.0	0.85	65.3	85.0	5.4	13.5	& 46.0
GB x R10	* 7.1	0.63	100.0	100.0	5.7	& 18.2	& 46.3
GB x R11	63.3	0.87	67.6	83.3	5.9	& 29.3	& 51.3
GB x R12	31.4	0.88	* 27.8	56.7	2.9	16.3	& 48.5
GB x R13	* 23.1	0.84	* 46.6	58.8	3.7	9.6	& 46.6
GB x R14	48.3	0.85	67.4	83.9	6.2	& 17.7	& 51.2
GB x R15	* 19.4	0.91	* 37.1	61.3	3.0	& 18.6	& 48.5
GB x R16	36.4	& 1.20	* 29.9	48.8	2.5	& 26.4	43.3
GU x R6	* 7.7	0.95	* 25.6	62.5	3.8	& 21.2	& 46.4
GU x R7	42.9	1.00	* 6.8	* 25.0	0.8	& 22.0	33.5
GU x R8	* 28.6	0.73	71.9	100.0	6.1	4.5	37.3
GU x R9	* 16.7	0.99	* 40.8	62.5	3.3	9.4	& 49.4
GU x R11	* 10.0	0.81	66.3	100.0	5.8	10.9	& 50.6
GU x R12	* 20.8	0.96	65.0	68.8	6.9	11.4	42.2
GU x R15	* 10.0	0.78	* 15.0	* 15.0	0.8	14.0	& 50.4
GU x R16	* 9.1	0.95	* 3.3	* 22.5	0.5	11.5	& 49.1
GU x R17	* 22.6	0.99	* 5.3	* 40.0	1.3	6.0	45.2
GU x R18	* 4.5	0.81	100.0	100.0	4.8	8.3	44.1
GU x R19	* 26.6	0.98	* 48.8	64.6	3.5	6.8	& 49.7
GU x R20	* 30.2	0.86	63.5	73.8	8.6	10.0	& 48.6
GU x R21	29.9	& 1.31	* 0.0	* 20.6	0.9	9.1	45.1
GU x R22	* 25.4	0.85	* 42.4	59.4	2.3	9.7	& 48.4
GU x R23	36.0	1.02	54.9	69.1	5.8	14.4	& 51.0
GU x R24	* 16.1	1.01	* 22.2	73.3	6.2	& 22.7	& 52.0
GU x R25	59.2	0.76	93.4	100.0	6.4	11.4	& 49.8
GU x R26	* 16.7	0.91	* 9.7	53.3	1.8	13.5	& 48.2
GU x R27	* 18.2	0.63	55.8	87.5	3.0	1.2	& 51.2
GU x R28	* 19.8	1.04	61.0	80.0	7.5	14.9	& 50.0
GU x R29	* 11.5	& 1.26	* 0.0	* 27.5	0.5	13.5	& 52.5
GU x R30	* 15.3	0.85	* 35.0	50.0	2.1	9.7	& 51.7
GU x R31	* 23.0	0.94	* 34.0	52.2	8.2	12.1	& 47.5
GU x R32	27.9	1.00	* 34.1	50.3	3.6	11.5	& 50.9
GU x R33	* 15.0	1.06	* 23.9	86.7	7.6	5.3	& 48.4
GU x R34	* 19.9	1.09	* 25.0	56.3	6.6	7.7	& 49.9
GU x R35	46.2	& 1.14	* 28.3	80.0	2.9	& 18.9	& 47.7
GU x R36	33.3	0.92	* 23.8	61.3	4.2	16.8	& 50.4
GU x R37	* 0.0	& 1.52	* 0.0	* 0.0	# 0.4	& 22.8	& 47.9
GU x R38	* 16.7	1.01	* 1.9	* 38.8	3.6	14.6	& 49.2
Cultivars							
ACA 884	* 28.1	1.08	* 28.6	48.8	2.7	13.4	42.8
PARAÍSO 20	* 13.6	& 1.16	* 3.6	97.5	13.3	12.4	& 47.1
VDH 487	* 18.2	0.99	* 7.6	* 23.3	0.6	12.6	& 52.4
General mean	23.8	1.0	38.4	61.9	4.2	13.7	48.0
CV (%)	66	17.1	63.8	31.5	70.6	41.4	6.5
$LSD_{0.05}$	30	0.39	54	43	---	11.8	6.6

[1]INC= Incidence; RIP= Relative Incubation Period; SEV-40= Severity at 40 days after inoculation; SEV-MX= Maximum Severity; WRP= White Rot Progress; SW= Seed Weight; SO= Seed Oil.
*Value equal to the minimum one in the variable, since the $LSD_{0.05}$ test; [&]Value equal to the maximum one in the variable, according to the $LSD_{0.05}$ test; [#] Rounded value.

The LSD value of 30% indicated that 30 test-crosses as well as the three cultivars were not significantly different from the GBxR8 and GUxR37 test-crosses, without symptoms in this experiment. In these 32 test-crosses the disease incidence was lower than in the lines GU and GB. Therefore, the R inbred-lines used must have contributed to reducing disease incidence in these test-crosses in relation to the testers. The inbred-lines R8, R9, and R15, were crossed to both testers (CMS GU and CMS GB), and their hybrids had similar disease incidence values to those of both check cultivars.

The relative incubation period of the GBxR8 and GUxR37 test-crosses, which showed no symptoms, was estimated according to Castaño et al. (1993). The calculated value (1.52) was 10% greater than the highest one (1.38) shown by GBxR1 in this experiment. General mean was 1.00 and range 0.89. Analysis of variance detected significant (α=0.02) effects of genotypes. The LSD value (0.39) detected 7 test-crosses and the cultivar PARAÍSO 20 with similar relative incubation period values to the maxima calculated for GBxR8 and GUxR37.

The general mean of the disease severity at 40 days after inoculation had a value of 38.4% and the range was 100%. Analysis of variance showed significant (α=0.01) differences between genotypes. The LSD value (54%) determined that 26 test-cross and all three cultivars had similar severity 40 dai values to the four following test-crosses: GBxR8, GUxR37, GUxR21, GUxR29, with the minimum (0%).

Maximum disease severity had a general mean value of 61.9% and a range of 100%. Analysis of variance indicated that genotype responses differed significantly (α=0,001). LSD value was 43%, determining that 8 test-crosses and the cultivar VDH 487 showed similar maximum disease severities as both GBxR8 and GUxR37 test-crosses without symptoms.

The daily white rot progress of two non diseased test-crosses was estimated in the same way as for the relative incubation period. The calculated value was 0.45, 10% less compared with the lowest value (0.5) observed for GUxR29 and GUxR16 in this experiment. Mean of daily progression of symptoms was 4.2% and the range was 12.8%. Unlike previous four white rot variables, the analysis of variance did not detect any differences between genotype responses (α=0.12). In spite of the high range of white rot progress values, the absence of different genotype responses could be related to the coefficient of variation of 70.6% showed by this variable, the highest one in the experiment.

Test-crosses obtained from the line CMS GU with the restorers R21, R29, and R37, as well as CMS GBxR8, showed favourable responses for white rot variables. These R inbred-lines contributed to increasing the level of resistance of all phases of the disease development in the test-crosses evaluated. Excepting R3, R20, R25, and R23, the inbred-lines showed advantageous effects for at least one phase of white rot development. The inbred-line R11 however showed a good performance with respect to disease incidence when it was crossed to CMS GU, but was very susceptible when it was crossed with CMS GB since this test-cross reached the maximum incidence value (63. 3%).

Seed weight and seed oil content

Mean value for seed weight was 13.7 g/cap and the range was 28.1 g/cap. The check VDH 487 had 12.6 g/cap, this value being lower than the average (52 g/cap) shown by these cultivars in 8 trials of the National Network of Sunflower in Argentina during 2006/07 (Quillehauquy et al., 2007). According to Hall et al. (1985), Ravishankar et al. (1991), and Nel et al. (2000) the absence of adequate environmental humidity would have decreased both the seed size and seed weight in this cultivar, but also in the other genotypes grown in this experiment. Analysis of variance showed significant (α=0.02) differences between genotypes. The LSD value of 11.8 g/cap showed that 10 test crosses had statistically similar seed weight to GBxR11, which was the best (29.3 g/cap). Three test crosses: GBxR11, GBxR16, GBxR6, showed significantly more seed weight than the check VDH 487.

General mean of seed oil content was 48% and the range was 19%. The fertile inbred lines GU and GB showed 48% and 44%, respectively (data not shown). The cultivar VDH 487 had a value of 52.4%, which was higher than the mean value (48%) shown by the same cultivar in 19 trials in the National Network of Sunflower in Argentina (Quillehauquy et al., 2007). According to Steer et al. (1988), the fact the seed size and seed weight were altered by the lack of adequate humidity could be related to the maintenance of seed oil content in this cultivar and other genotypes in this experiment. Analysis of variance detected significant (α=0.01) differences between genotypes. The LSD value was 6.5% and it determined that 37 test-crosses as well as cultivars VDH 487 and PARAÍSO 20 had similar seed-oil percentage as the maximum (52.5%) shown by GUxR29. All these test-crosses, excepting GUxR6, GUxR31, GUxR35, and GUxR37, showed higher seed-oil values than the fertile inbred-lines GU and GB, whose male-sterile version were the female in the test-crosses. This effect suggests a favourable contribution of R inbred-lines in the seed oil content in these test crosses.

Relationship between white rot resistance and seed-weight and oil content of healthy plants

The association between genotype responses to *S. sclerotiorum* inoculations and the agronomic attributes measured in plants without symptoms was calculated and the correlation coefficients are shown in Table 2.

Table 2. Correlation coefficients, linear (r) and rank (r_s, in italics), between white rot variables and seed weight and seed oil content of plants without symptoms

	Incidence	Relative incubation period	SEV-40dai	SEV- MX
Seed-Weight	0.26	0.21	-0.04	-0.08
Seed-Oil	*0.04*	0.04	*0.09*	*0.06*

Coefficients of linear correlation varied between r= 0.26 and r= -0.08, while those of rank correlation oscillate between r_s= 0.09 and r_s= -0.04. None of the coefficients were statistically different from zero (n=49, p> 0.05). In this experiment, the level of disease resistance in genotypes could be considered as being independent of the seed weight and seed oil content measured on healthy plants.

The apparent absence of effects of *S. sclerotiorum* resistance on the agronomic traits measured would suggest that the level of white rot resistance does not have any substantial cost, from a biological point of view, for the sunflower genotypes when the disease is absent.

This characteristic is not exclusive of the sunflower-*Sclerotinia* interaction, since the bibliography reports similar results with other crops and pathogens. For example, Miles et al. (1980) and St. Martin et al. (1994) found that in environments without disease the seed yield of genotypes with a higher level of resistance was not reduced compared with that shown by less resistant genotypes in maize-*Heminthosporium turcicum* and soybean-*Phytophtora infestans* pathosystems, respectively.

Development of cultivars with high levels of resistance implies selecting genotypes with the best disease performance. These genotypes must be adapted for use in environments with disease risk. Then, if the disease appeared, resistant cultivars will have a better agronomic behaviour than susceptible or non-adapted ones (Brown, 2002).

In this sense, Creus et al. (2007) showed that resistant sunflower isohybrids to *Verticillium dahliae* yielded 30% more than the homologous susceptible ones, when Verticillium wilt was present. In the available bibliography, there does not appear to have been any similar work using white rot disease, but it can be assumed that genotypes with a higher level of resistance yield more than susceptible ones when white rot occurs.

Sunflower breeders consider many factors, such as white rot resistance for example, when deciding whether or not to release a hybrid. The fact that the resistance to *Sclerotinia* infection on capitula does not condition the seed weight and seed-oil content in non diseased test-crosses would allow the possibility of combining adequate levels of white rot resistance with high seed weight and seed-oil content in the same genotype. If these selected cultivars were used, the agronomic stability of sunflower crop could be improved given that the seed weight and seed oil content oscillations between environments with and without white rot appearance could be diminished.

CONCLUSIONS

Because the results were obtained from only one experiment they must be considered as being preliminary ones. A further trial will allow an estimation of their repeatability. In this first experiment it could be concluded that:

1- Genotypes were diversified for all white rot variables, except daily white rot progression.

2- 72% of test-crosses (33/46) had similar disease incidence values as the checks ACA 884 and PARAÍSO 20. In these test-crosses, the R inbred-lines contributed to reduction of disease incidence compared with testers.

3- There were some R inbred-lines with favourable resistance contribution for at least one phase of the disease development.

4- There was variability in seed weight and seed oil content in genotypes not inoculated with *S. sclerotiorum*.

5- Test-crosses produced by the inbred-lines R6, R11, and R16 with CMS GB, had higher seed weight than the check VDH 487. Those obtained after crossing the inbred-lines R6, R31, R35, and R37 with CMS GU, had similar oil contents to those of the check but higher than the tester.

6- The level of white rot resistance did not restrict the seed weight and seed oil content when the disease was absent in the genotypes evaluated

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