Heterosis and the level of white rot resistance at early stages of development in sunflower

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ABSTRACT

- In all humid and temperate cultivated areas, *Sclerotinia sclerotiorum* attacks on sunflower capitula produce white rot. Our group determined that white rot is a disease composed of different phases. Sunflower breeders develop cultivars showing hybrid vigor. Literature does not show references about the presence of heterosis for white rot resistance. The aim is to assess the superiority of F1 hybrids in relation to the average of parental lines for the level of resistance at early stages of white rot development.

- Two field experiments were carried out in a location. In the first 49 F1 hybrids of a factorial mating design were grown. In the second the 14 parental inbred lines were placed. Assisted infections were made since the current protocol. Two components of partial resistance, incidence (DI) and the relative incubation period (RIP) of white rot were quantified.

- Analysis of variance detected different behaviors of inbred lines and F1 hybrids for both variables. Midparent heterosis effects were significant and favorable in 13% of F1 hybrids evaluated by DI and in 38% of them evaluated by RIP.

- Despite further studies are needed, it is showed that level of sunflower resistance at early stages of white rot may be increased from mid-parent behavior. Hybrid vigor in hybrids may be therefore assessed as well as included in the sunflower breeding strategy for white rot resistance.

- Hybrid testing in different environments must be then necessary to corroborate the hybrid vigor of selected hybrids at field conditions.

Key words: breeding, disease resistance, hybrid vigor, *Sclerotinia*, selection

INTRODUCTION

Argentina is a very important producer of sunflower seeds. The southeast of Buenos Aires Province is the main seed harvested area in our country. In all humid and temperate cultivated areas, like the southeast of Buenos Aires Province, *Sclerotinia sclerotiorum* attacks on sunflower capitula produce white rot, a disease which is responsible direct of both seed-yield and seed-oil losses. Previous works determined that the white rot may be considered as a disease composed of different phases of a whole cycle beginning at flowering and ending at crop maturity.

The inheritance of white rot resistance is of quantitative type (Castaño et al. 2001) and different components allow identifying genotypes with adequate level of the partial resistance (Castaño & Giussani 2009). Two components are well known and they are related to the resistance to the fungal penetration into the inflorescence and the resistance to early apparition of symptoms. In the practice, they are valuated as the white rot incidence (DI) and the relative incubation period (RIP), respectively (Castaño et al. 1993).

Sunflower F1 hybrids are the first generation progeny of a cross between selected and genetically diverse parents (i.e. female A-line and male R-line). In this type of cultivar the hybrid vigor (i.e. heterosis) is exploited. The heterosis is one of the major achievements in plant breeding as well as the primary reason for the success of the commercial sunflower industry.

Literature shows several evidences that, for seed-yield, the hybrid vigor appears in sunflower hybrids after crossing selected inbred lines. However, none references were found regarding the presence of heterosis for other quantitative inherited character like the white rot resistance. Its knowledge may help the sunflower breeding strategy of hybrids to be grown in areas with white rot apparition risk like the southeast of the province of Buenos Aires, Argentina. The selection of F1 hybrids showing heterosis for low DI and high RIP may have better behavior at the beginning of white rot development than those ones without hybrid vigor effect.

Therefore, the aim of this work is to assess the potential superiority of single F1 hybrids in relation to the average of parental lines for the level of resistance at early stages of white rot development.

MATERIALS AND METHODS

Two field experiments following a randomized complete block design with three replications were carried out in Camet, a location placed in the southeast of Buenos Aires Province. In the first experiment, 49 single F1 hybrids obtained from a 7 female A-lines x 7 male R-lines of a factorial mating design were grown, whereas in the second the 14 parental inbred lines were involved.

The main inflorescence of R-lines and the unique capitulum of A-lines as well as the one of F1 hybrids received once the aqueous suspension containing the pathogen's ascospores at the R5.2 stage (Schneiter and Miller 1981) (= F3.2, Cetiom 1992). Because of seed germination problems in the F1 hybrids ADK5xR199 and ADK3xR200, only 47 out of the 49 hybrids were inoculated. Immediately after infection, the infected capitula were covered with Kraft paper bags. There were regular irrigations by sprinklers. Inoculated capitula were evaluated twice a week looking for white rot symptoms. Two components of partial resistance, the incidence (%) and the relative incubation period of white rot, were quantified as follow.

The disease incidence is the percentage of inoculated plants/plot showing white rot symptoms at the R10 stage (Schneiter and Miller 1981) (=M4, Cetiom 1992). If the disease incidence decreases, the level of resistance of genotypes to *Sclerotinia* penetration on capitulum increases. The relative incubation period is the ratio of the number of days between infection and first symptom appearance for the capitula and that for the check. A relative incubation period value higher than one suggests that the rate of *Sclerotinia* mycelia extension in adult tissues of genotypes' capitula was lower than the check. Therefore these genotypes showed higher level of resistance to the extent and growth of *Sclerotinia* mycelium than the check.

Two analyses of variance with two main effects each (e.g. inbred lines or F1 hybrids as fixed effect and replicates as random) were performed following Steel and Torrie (1988). The LSD test was used and three groups of different levels of resistance were constituted. Two of them contained inbred lines or F1 hybrids in which estimated disease values were statistically similar to either the maximum or the minimum disease values obtained in the experiments. Group 1 (G1), with the lowest level of resistance in this experiment, included genotypes whose DI values were statistically similar to the maximum one or

RIP values were similar to the minimum one obtained in the experiments. The G3, with the highest level of resistance, had inbred lines or F1 hybrids showing DI or RIP values similar to the minimum or the maximum values, respectively. Genotypes placed in the G2 group differed from those ones having the maximum and minimum disease values and they showed a level of resistance intermediate between G1 and G3

Midparent heterosis effects (H) were calculated, agreeing Hallauer and Miranda (1988), as H= (F1-MP)/MP. Herein, F1 is the F1 hybrid mean whereas MP is the average mean of the two parental inbred lines. The H effects may show either positive or negative sign. If H value for DI was negative, the F1 hybrid had lower (i.e. favorable effect) proportion of diseased capitula that the mean of two inbred parental lines. For RIP, a favorable F1 hybrid behavior was with a positive H value. Standard errors (SE) for H effects of the F1 hybrids were calculated by methods described by Sing and Chaudhary (1977). Tailed t test was performed to test the significance of the H effect, where t= H/SE.

RESULT AND DISCUSSION

Inbred lines showed an experimental mean value of 75% (incidence) and of 0.78 (relative incubation period) (Table 1) whereas the average of F1 hybrids reached 67% (incidence) and 0.96 (relative incubation period) (Table 2). The coefficients of variability ranged between 10.4% and 21%. These values are considered adequate when these attributes are analyzed.

Table 1. Disease incidence (DI) and relative incubation period (RIP) of 14 sunflower inbred lines grown in Camet.

Inbred lines	DI (%)	RIP
R122	100	1.14
R152	94	0.72
R161	100	0.65
R167	60	0.52
R199	8	0.89
R200	50	0.87
PAC1	100	1.03
ADK1	84	0.74
ADK2	77	0.82
ADK3	50	0.33
ADK4	85	0.65
ADK5	100	0.79
ADK6	64	0.97
SD	73	0.78

Table 2. Disease incidence (%), in brackets, and relative incubation period of F1 hybrids after *Sclerotinia* artificial infections on capitula

Female lines	ADK1	ADK2	ADK3	ADK4	ADK5	ADK6	SD
Males lines							
R122	[98] 0.66	[97] 0.75	[92] 0.77	[96] 0.80	[100] 0.58	[69] 0.91	[83] 0.89
R152	[100] 0.84	[37] 0.88	[40] 1.11	[44] 0.98	[95] 0.77	[55] 1.00	[7] 1.45
R161	[83] 1.01	[44] 0.99	[33] 0.93	[69] 1.06	[96] 0.77	[52] 1.05	[43] 1.10
R167	[96] 0.82	[70] 0.99	[62] 1.01	[74] 1.14	[100] 0.73	[52] 1.01	[38] 1.10
R199	[83] 0.90	[40] 1.02	[26] 1.24	[62] 0.98	//	[28] 1.12	[22] 0.96
R200	[100] 0.86	[88] 0.90	//	[91] 0.98	[100] 0.79	[70] 0.98	[77] 0.99
PAC1	[85] 0.90	[46] 1.04	[72] 1.13	[33] 1.13	[96] 1.03	[79] 1.06	[25] 1.00

The dispersion of DI and RIP values was also calculated. In inbred lines the disease incidence ranged between 8% (R199) and 100% (R122, R161, PAC1, ADK5), whereas in F1 hybrids between 7% (SDxR152) and 100% (ADK1xR152, ADK1xR200, ADK5xR122, ADK5xR167, ADK5xR200). When the relative incubation period was considered, the variation of values was from 0.33 (ADK3) to 1.14 (R122) and from 0.58 (ADK5xR122) to 1.45 (SDxR152) in inbred lines and F1 hybrids, respectively.

Analysis of variance detected highly significant effects ($p < 0.01$) of inbred lines as well as of F1 hybrids for both components of resistance. This suggests the variability of reactions against *S. sclerotiorum* attacks and therefore that both evaluated inbred lines and F1 hybrids had differential responses when they were evaluated by the disease incidence and the relative incubation period of white rot.

A LSD value of 26 % indicates that the behavior of the lines ADK1, ADK2, ADK4 and R152 were not different from those showing the maximum (100%) DI value. They were placed in the G1 group. The line R199 (8%) was the only at the G3 group. The remaining five lines (i.e. R167, R200, ADK3, ADK6, SD) were at the G2 group. For RIP, the LSD value was 0.18 and it determines that the G1 group comprised only the line ADK3 (0.33). The G3 group grouped PAC1 and ADK6 whose RIP values were similar to the maximum (1.14) showed by R122. The remaining 10 inbred lines (i.e. R152, R161, R167, R199, R200, ADK1, ADK2, ADK4, ADK5, SD) were in the G2 group.

Females	ADK1	ADK2	ADK3	ADK4	ADK5	ADK6	SD
Males							
R122	[6] -0.28	[-3] -0.23	[17] 0.03	[4] -0.10	[0] -0.38	[-13] -0.15	[-3] -0.08
R152	[11] 0.11	[-49*] 0.12	[-32] 0.59*	[-45*] 0.30	[-2] 0.02	[-24*] 0.15*	[-76*] 0.70*
R161	[-9] 0.31*	[-45*] 0.26	[-42] 0.44*	[-24] 0.41*	[-4] 0.06	[-30] 0.23*	[-43] 0.39*
R167	[25] 0.19*	[1] 0.32*	[7] 0.58*	[1] 0.55*	[20] 0.07	[-10] 0.27*	[-28] 0.45*
R199	[37*] 0.09	[-2] 0.17	[-3] 0.64	[16] 0.21*	//	[-8] 0.19	[-18] 0.12

The LSD test showed a value of 23% when F1 hybrids were evaluated by DI. The G1 group had 20 hybrids of which five of them showed the maximum DI value (100%). Another four hybrids had DI values similar to the minimum (7%) and these five F1 hybrids constituted the G3 group. The remaining 22 hybrids were in the G2 group. A LSD test value of 0.16 allowed detecting two hybrids (i.e. ADK1xR122, ADK5xR167) with RIP values similar to the lowest one and they formed the G1 group. The G3 group had only one hybrid (SD×R152), which it showed the maximum value (1.45). The remaining 43 hybrids were in the G2 group.

Table 3 shows the midparent heterosis effects calculated for the 47 sunflower F1 hybrids in relation to the average behavior of their parental lines. For disease incidence, midparent heterosis ranged between -76% (SDxR152) and 37% (ADK1xR199) and the general mean reached a value of -9%. This suggests that the F1 hybrids showed together a lower proportion of white rotted plants than the parental line mean. According to the "t" test, heterosis effects were significant ($p < 0.05$) as well as favorable in 13% of evaluated F1 hybrids.

Table 3. Midparent heterosis effects in F1 hybrids evaluated by white rot incidence (in brackets) and relative incubation period.

R200	[33] 0.05	[24] 0.06	//	[23] 0.22*	[25] -0.04	[13] 0.06	[16] 0.16
PAC1	[-7] 0.12	[-43] 0.12	[-3] 0.45*	[-59*] 0.29*	[-4] 0.12	[-3] 0.07	[-61*] 0.09

<u>Note:</u> * significant at p<0.05

When the relative incubation period is considered, the midparent heterosis effects were between -0.38 (ADK5xR122) and 0.70 (SDxR152). The average of midparent heterosis effects was 0.18. It indicates that first white rot symptoms appear later in F1 hybrids than in the average of their inbred lines. The 38% of F1 hybrids showed significant (p<0.05) and favorable heterosis effects for this variable.

In order to know the possibility of predicting the midparent heterosis effects through parental line responses, the relationship between the inbred line *per-se* behavior and the average of midparent heterosis effects of their progeny was estimated. The correlation coefficients reached values of r= -0.39 (p>0.05) and r= -0.79 (p<0.001) for white rot incidence and relative incubation period, respectively. These coefficients suggest a significant and indirect general relationship between the *per-se* behavior of inbred lines and the heterosis effect of their progeny. The midparent heterosis effects of F1 hybrids evaluated by the relative incubation period may be predicted because of the inbred lines showing early white rot symptoms (i.e. short incubation period) generated an averaged progeny with latter apparition of first white rot symptoms than their parental lines. This is due to a high difference between the values of the F1 hybrids and of the parental mean in the calculating formula of midparent heterosis effect.

Spearman's rank correlation was used as a measure of the linear relationship between the ranked data of F1 hybrid behaviors (Table 2) and the midparent heterosis effects (Table 3). The rank coefficients were r_s= 0.73 (i.e. DI) and r_s= 0.79 (i.e. RIP), both highly significant (p <0.01) different from zero. The two correlation coefficients were positive and it indicates that the ranks of both variables increased generally together. That suggest that F1 hybrids with high and favorable midparent heterosis effects for DI or RIP were those with good behavior at the beginning of white rot development, and vice versa.

We know heterosis as a phenomenon related to the level of heterozygozity in hybrids and as a function of the dominance in those loci with different alleles in the inbred lines. In our study, we assume allelic variability because parental lines of two different sunflower groups (i.e. A and R inbred lines) were used (Vear and Miller, 1993). The presence of non-additive gene effects is showed by the deviation of F1 hybrids from the mean values of their parental inbred lines. Several disease resistances, like white rot in sunflower, are quantitatively inherited and therefore controlled by several loci or QRLs that adding their resistance effects (Castaño et al., 2001). Despite further studies are needed, it is showed that level of sunflower resistance at early stages of white rot may be increased from mid-parent behavior. Thus we suggest that hybrid vigor in hybrids may be assessed as well as included in the sunflower breeding strategy for white rot resistance.

The selection of sunflower inbred lines having different alleles at loci regulating resistance at early stages of white rot may contribute to hybrid behavior in environments with disease apparition risk. Therefore, good selection progress could be expected by exploiting heterosis by crossing adequate inbred lines. Nevertheless, hybrid testing in different environments must be necessary to corroborate the hybrid vigor with an adequate level of resistance of selected hybrids at field conditions.

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