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Analysis of genetic determination of partial resistance to white rot in sunflower

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Abstract: Sunflower is usually affected by white rot (WR), a disease produced by *Sclerotinia sclerotiorum*. Thus, breeders select WR resistant hybrids by means of field experiments replicated in different environments. The WR selection will be effective when the correlation between the phenotype and the set of genes controlling the trait is high. This study aimed to estimate the relationship between the genotype and phenotype for components of WR partial resistance in hybrids. Also, the genotypic merit of these hybrids is estimated to determine their value in breeding programs. To this end, 37 cultivars were used during three years in Balcarce (southeast of Buenos Aires Province, AR). Plants were inoculated with *S. sclerotiorum* in their capitula. The WR variables evaluated were the relative incubation period (RIP), the daily lesion growth (DLG) and the relative DLG. By using transformed data, the degree of genetic determination (DGD) reached values of 0.78 (RIP), 0.63 (relative DLG) and 0.35 (DLG). Although all error variances and their relative contributions to the total variance had the highest values, the DGD values for RIP and relative DLG were higher than those reported in the bibliography. The best linear unbiased predictors (BLUPs) detected six hybrids with most suitable genetic merit for RIP and relative DLG. The BLUP correlation coefficient suggested that resistance genes involved in RIP and relative DLG were not the same. Thus, these genes could be used simultaneously to develop new sunflower hybrids with more complex WR resistance.

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Introduction

In Argentina, more than 50% of sunflower (*Helianthus annuus* L.) seeds are produced in the south and southeast regions of Buenos Aires Province, where the soil and climate are appropriate for the occurrence of *Sclerotinia sclerotiorum* infections in capitulum, also known as white rot (WR) (Castaño 2018). Thus, to reduce potential seed-yield losses and annual seed-production oscillations, farmers of these regions, as well as farmers of other regions worldwide where this pathogen occurs, must use hybrids with moderate resistance to WR.

WR resistance is of horizontal type (Castaño et al. 2001) and can be affected by environmental conditions that modify the genotype-phenotype correlation (Godoy et al. 2005). Thus, the field selection of moderately resistant hybrids is also altered. After assisted inoculations, WR resistance can be evaluated through variables like the relative incubation period (RIP) (Vear and Tourvieille 1988), as well as by the daily lesion growth (DLG) (Castaño and Giussani 2006).

Suitable hybrids have to be selected through phenotypic field observations in different environments (Castaño and Giussani 2009). This selection will be effective when the relationship between the phenotype and the set of genes (i.e. genotype) controlling the level of WR resistance is high and direct. Previous studies have shown a relationship between the values of genotypic variance and those of the phenotypic variance for RIP. In Argentina, Godoy (2001) detected that the genotypic contribution to phenotypic variance in the 49 hybrids evaluated in two localities of southeastern Buenos Aires was of 67%, whereas Filippi et al. (2017) showed a contribution of 0–48% (annual) and of 11% (pooled across five years) when 69-137 B and R-lines were tested in Balcarce. Regarding DLG, the only study so far performed showed a relationship between the genotypic and phenotypic variances of 54% when 31 hybrids were evaluated in Balcarce (Castaño and Giussani 2006). Given the variability of values, new estimations of genotypic contribution to phenotypic variance by means of the degree of genetic determination (DGD) could be made using other germplasm, environments and calculation methods. Thus, a comparison with those results could be done.

The best linear unbiased predictor (BLUP) of random effects, also known as BLUP, is a very popular methodology because the genetic merit of genotypes is

determined with the minimum error (Bernardo 1994). In sunflower, BLUPs have allowed detecting the best hybrids for seed-yield (Reif et al. 2013) and seed-oil content (Mangin et al. 2017). However, to our knowledge, no BLUP studies have been performed before for WR resistance. BLUPs could allow detecting favorable WR-resistant hybrids as well as evaluating the relationship between different WR variables.

Thus, in the present study we estimated the DGD, the BLUPs of hybrids, and the genetic correlation between WR-resistant variables.

Materials and methods

Plant material and experimental design

Thirty-seven cultivars (33 single and four three-way hybrids) were used. All were commercialized in the south and southeast of Buenos Aires Province. Hybrids were sown in Balcarce from 2010 to 2012, following a randomized complete block design with three replications. The hybrids Paraíso20 and ACA884, used as flowering checks, were adjacent to the experimental designs. Plots had around 20 plants each.

Inoculation and WR variables measured

Ascospores of *S. sclerotiorum* were from sclerotia collected the year before inoculation from naturally infected capitula. Inoculations were made following Vear and Tourvieille (1988). Thus, 12 plants in R5.3 stage (Schneiter and Miller 1981) or its homologous F3.2 (Martin-Monjaret 2019) were chosen from each plot and their capitula were sprinkled once with an aqueous suspension containing around 25,000 ascospores. Capitula were covered with paper bags, which were left until the end of the experiment. Given the variability of flowering dates both within and between plots, there were 3–4 inoculation dates for each year. Daily irrigations were up to 2 mm.

From the 14th inoculation date, one person evaluated each capitulum twice a week by touching it through the paper bags, until WR symptoms were detected. Thus, diseased capitula were examined every seven days until physiological maturity. The date and WR severity (i.e., the relationship between the WRten area and the total capitulum surface) were scored at each evaluation.

In diseased capitulum, we estimated: (1) the RIP, i.e., the relation between the incubation period of the inoculated capitulum and the average of periods of the checks inoculated on the same date; (2) the DLG, in % day^{-1}, i.e., the linear regression coefficient of daily WR severity progress (from first detected WR symptoms until the maximum WR severity reached); (3) and the relative daily growth lesion (RDLG), i.e., the relation between the DGL in the capitulum and that of the mean of the checks inoculated on the same date. Hybrids with favorable level of resistance have RIP > 1, DLG < 1 and/or RDLG < 1.

Statistical analyses

The results were analyzed by using the following model:

$$y_{ijks} = \mu + \alpha_i + \gamma_j + \beta_{k(j)} + (\alpha\gamma)_{ij} + \varepsilon_{ijk} + \delta_{ijks}$$

where: y_{ijks}: response of the ith hybrid, in the jth year, in the kth block of the jth year and in the sth diseased plant; μ: general average; α_i: effect of the ith hybrid; γ_j: effect of the jth year; $\beta_{k(j)}$: effect of the kth block within the jth year; $(\alpha\gamma)_{ij}$: effect of the interaction between the ith hybrid and the jth year; ε_{ijk}: error associated with the ijkth plot; and δ_{ijks}: error of the $ijks$th diseased plant. All effects were assumed as random.

When the variables were not normally distributed and homoscedastic, the Box and Cox parameter (λ) was calculated. For original and transformed variables, models were adjusted with the "lmer" function in the "lme4" package for R (R Core Team 2013). The Restricted Maximum Likehood (REML) method was used to estimate the components of the variances and to test their null hypothesis equal to zero.

Genetic analyses

From the adapted equation for unbalanced data of Holland et al. (2003), the DGD was estimated as:

$$\widehat{DGD} = \frac{\widehat{\sigma}_g^2}{\widehat{\sigma}_g^2 + \frac{\widehat{\sigma}_{gy}^2}{y} + \frac{\widehat{\sigma}^2}{yr} + \frac{\widehat{\sigma}_w^2}{yrp}}$$

where: $\widehat{\sigma}_g^2$: genetic variance; $\widehat{\sigma}_{gy}^2$: hybrid-year interaction variance; $\widehat{\sigma}^2$: plot-to-plot error variance; $\widehat{\sigma}_w^2$: within-plot error variance (among WR-diseased plants); y: years; r: harmonic mean of replications/hybrid; p: harmonic mean of WR-diseased plants/hybrid.

Finally, the genotypic value of hybrids was estimated through BLUP. The t-test was used to determine whether each BLUP value was significantly different from the mean of BLUPs for each variable.

Results

Inter-annual variability of WR variables

The maximum general averages of RIP and RDLG (1.07 and 1.22, respectively) were observed in 2011, whereas the minimum ones (0.90 and 0.85, respectively) were observed in 2012. The maximum general average of DLG (7.26% day^{-1}) was observed in 2010, whereas the minimum one (4.69% day^{-1}) was observed in 2012 (Table 1).

Components of phenotypic variance and degree of genetic determination

Data of RIP, DLG and RDLG were not normally distributed and the Box and Cox parameters were $\lambda = 0.5$ (RIP) and $\lambda = 0.25$ (DGL and RDGL). Combined analysis of

Table 1: Annual general averages and standard deviations of white-rot variables assessed in sunflower hybrids evaluated during three years in Balcarce.

Variables[a]	RIP	DLG (% day^{-1})	RDLG
Years			
2010	0.99 ± 0.20	7.26 ± 3.22	1.07 ± 0.47
2011	1.07 ± 0.25	4.92 ± 2.77	1.22 ± 0.68
2012	0.90 ± 0.30	4.69 ± 3.29	0.85 ± 0.53

[a]RIP: Relative incubation period; DLG: Daily lesion growth; RDLG: Relative daily lesion growth.

variance detected significant effects ($p < 0.01$) of years, blocks/year, hybrids and hybrid-year interaction for all transformed variables (i.e., RIPt, DLGt, RDLGt).

The error variances (between + within-plots) always showed the highest values (RIPt = 0.047, DLGt = 8.431, RDLGt = 0.234) and relative contributions to the total variance of field observations (RIPt = 66.2%, DLGt = 73%, RDLGt = 75.5%) (Table 2). For RIPt, the variance of hybrids (0.010) and its relative contribution (14.1%) were immediately ranked below, with values higher than those of the hybrid-year interaction variance (0.006) and of its relative contribution (8.4%). In contrast, for DLGt and RDLGt, the hybrid-year interaction variances and their relative contributions were higher than those for hybrids (Table 2).

The estimated DGD values of 0.78 (RIPt), 0.35 (DLGt) and 0.63 (RDLGt) indicated the contribution of the genotypic variability to phenotypic diversity among hybrids for each WR variable.

Table 2: Estimated components of variance and their relative weights (%) to the total variance of RIPt, DLGt and RDLGt.

	RIPt[a]		***DLGt***		***RDLGt***	
	$\widehat{\sigma}^2$	%	$\widehat{\sigma}^2$	%	$\widehat{\sigma}^2$	%
Sources of variation						
Years-Y	0.007	9.9	1.907	16.5	0.038	12.2
Replications\Y	0.001	1.4	0.039	0.3	0.003	1.0
Hybrids-H	0.010	14.1	0.240	2.1	0.017	5.5
HxY	0.006	8.4	0.935	8.1	0.018	5.8
Between-plots error	0.006	8.5	0.516	4.5	0.015	4.8
Within-plots error	0.041	57.7	7.915	68.5	0.219	70.7

[a]RIPt: Transformed relative incubation period; DLGt: Transformed daily lesion growth; RDLGt: Transformed relative daily lesion growth.

Genotypic value of hybrids and association between WR variables

Twenty hybrids showed positive BLUP values for RIPt (Table 3). Among them, Paraiso75 (0.16), DM220AO and SPS3109 (0.14), Paraiso27 (0.12) and Paraiso65 (0.10) showed significant ($p < 0.05$) values. Thus, these hybrids had the most favorable levels of resistance because of their longer relative period without WR symptoms after inoculation. In contrast, the hybrids ACA885, ACA863, Tobsol3004, ACA886DM and Paihuén showed the most unfavorable levels of resistance because of their significantly ($p < 0.05$) negative BLUP values.

Nineteen hybrids showed negative BLUP values for RDLGt, but only that of DM230 (−0.21) was significant ($p < 0.05$) (Table 3). Thus, it showed the lowest WR severity progress and the highest level of resistance to this variable. In contrast, TehuelcheCL and BuckSurcoflor showed unfavorable levels of resistance because of their positive and significant ($p < 0.05$) values.

An estimated coefficient of correlation of $r = 0.04$, not different from zero ($p > 0.05$), determined that the BLUPs of RIPt and RDLGt were not associated.

Discussion

The general average of WR incidence (i.e., percentage of WRted plants in relation to those inoculated/plot) was 92% and annual means ranged from 84.3 to 96.7%. These high incidence values can be attributed to high concentration of *S. sclerotiorum* acospores at inoculation as well as to inoculated capitula remained covered with bags until the end of the experiment. So, the potential level of resistance of hybrids to infection could have masked. The minimum annual mean was still a favorable value because RIP, DLG and RDLG were estimated from at least 30 diseased capitula/year, except for Paraiso75, DM220AO and Paraiso65 (Table 3), and this is within the range of values suggested to make accurate RIP estimations (Castaño et al. 1993).

The variability of RIPt, DLGt and RDLGt between years (Table 2) can be related to the heterogeneous meteorological conditions and to the use of different *S. sclerotiorum* isolates. Conditions of high relative humidity and relatively low temperature at flowering have been reported as favorable for WR incidence (Masirevic and Gulya 1992). Also, conditions of high relative humidity have been associated with the natural occurrence of WR epiphytes in the south and southeast regions of Buenos Aires during 1988 and 1998 (Moschini et al. 2002). In agreement with these results, in the present study, the maximum annual mean of WR incidence was observed in the year that presented temperatures of 18 °C and relative

Table 3: Estimated BLUPs of sunflower hybrids for RIPt and RDLGt, variance of predictors and *p*-values associated with *t*-tests.

Seed-Co.[a]	Hybrids	*df*[b]	RIPt			RDLGt		
			BLUP	$\widehat{\sigma}^2 \cdot 10^{-2}$	*p*	BLUP	$\widehat{\sigma}^2 \cdot 10^{-2}$	*p*
(1)	Paraíso75	75	0.16	0.27	<0.01	−0.06	0.70	0.48
(2)	DM220AO	46	0.14	0.32	0.02	0.18	0.84	0.06
(3)	SPS3109	93	0.14	0.25	<0.01	−0.04	0.66	0.62
(1)	Paraíso27	91	0.12	0.25	0.03	0.11	0.66	0.18
(1)	Paraíso65	89	0.10	0.25	0.04	−0.01	0.67	0.87
(4)	Albisol2	98	0.09	0.25	0.08	0.04	0.65	0.62
(5)	Macon	103	0.08	0.25	0.12	0.11	0.65	0.18
(1)	Paraíso22	99	0.08	0.25	0.10	−0.05	0.65	0.56
(6)	GS3190RDM	96	0.07	0.25	0.15	−0.15	0.66	0.07
(7)	Cauquen	90	0.06	0.26	0.23	−0.04	0.67	0.64
(8)	ACA884	99	0.05	0.25	0.34	−0.12	0.65	0.14
(9)	BuckSurcoflor	101	0.05	0.25	0.34	0.18	0.65	0.03
(3)	Dekasol3820	99	0.05	0.25	0.29	−0.06	0.65	0.47
(10)	TehuelcheCL	100	0.05	0.25	0.31	0.17	0.65	0.04
(1)	Paraíso20	96	0.04	0.25	0.39	−0.00	0.66	0.98
(11)	64A89	90	0.03	0.25	0.61	−0.12	0.66	0.14
(12)	MG60	104	0.03	0.25	0.55	−0.05	0.65	0.58
(13)	Agrobel963	103	0.02	0.25	0.76	−0.05	0.65	0.57
(14)	KWSBaqueano	102	0.02	0.25	0.70	−0.03	0.65	0.67
(15)	Pan7031	100	0.01	0.25	0.90	−0.06	0.65	0.45
(3)	Dekasol3845	106	−0.01	0.25	0.82	0.14	0.65	0.08
(16)	CF31	102	−0.02	0.25	0.74	0.07	0.65	0.42
(2)	DM230	95	−0.02	0.25	0.71	−0.21	0.66	0.01
(12)	MG2	100	−0.02	0.25	0.71	−0.06	0.65	0.44
(4)	Albisol20	98	−0.06	0.25	0.21	0.10	0.66	0.23
(16)	VDH487	101	−0.06	0.25	0.21	0.01	0.65	0.91
(14)	HO25AO	101	−0.07	0.25	0.16	0.03	0.65	0.75
(16)	HS-03	105	−0.07	0.25	0.19	0.04	0.65	0.67
(5)	NK70	95	−0.07	0.25	0.17	0.04	0.66	0.62
(7)	PamperoDM	105	−0.08	0.24	0.11	−0.14	0.65	0.09
(11)	65A25	101	−0.09	0.25	0.07	0.14	0.65	0.08
(17)	NTO3.0	101	−0.09	0.25	0.06	0.06	0.65	0.48
(18)	Paihuen	102	−0.10	0.25	0.04	−0.12	0.65	0.13
(8)	ACA886DM	102	−0.11	0.25	0.02	−0.16	0.65	0.05
(19)	Tobsol3004	100	−0.14	0.25	<0.01	0.03	0.65	0.70
(8)	ACA863	107	−0.15	0.25	<0.01	0.13	0.65	0.11
(8)	ACA885	104	−0.21	0.25	0.00	−0.03	0.65	0.73

[a]Seed *Co.* [1]Nidera; [2]Pau Seeds; [3]Monsanto Argentina; [4]Riestra Semillas; [5]Syngenta Agro; [6]SPS Argentina; [7]El Cencerro; [8]Asociación Cooperativas Agrarias; [9]Buck Semillas; [10]Clasificaciones Murphy; [11]Pioneer Argentina; [12]Mycogen; [13]Seminium; [14]KWS Argentina; [15]Pannar RSA; [16]Advanta Semillas; [17]Dow AgrSci. Argentina; [18]Ducos e hijos; [19]Tobin.

[b]*df*: degrees of freedom, $\widehat{\sigma}^2 \cdot 10^{-2}$: Blup variance of hybrids, *p*: *p*-values.

humidity of 75% on the inoculation dates, whereas the minimum was observed in the year that presented temperatures of 23 °C and relative humidity of 65%.

According to Robinson (2007), the possibility that there have been no genetic changes in the parasitic ability of the pathogen must have been assured by the use of different isolates. In addition, the ability of isolates to induce WR may vary (Castaño et al. 2001). However, given that we did not measure the aggressiveness of isolates in our trials, we assumed that isolates might have also contributed with the inter-annual variability.

In this study, the relative contribution of the hybrid-year interaction variance to the total variance of field observations (i.e., total sum of squares) ranged from 8.4 (RIPt) to 5.8% (RDLGt) (Table 2). To our knowledge, there was no study related to this type of contribution in experiments evaluating disease resistances in sunflower. In Germany, Degener et al. (1999) evaluated the components of the phenotypic variance of 85 inbred-lines after *S. sclerotiorum* stem inoculations in different environments and estimated a genotype-environment variance value ($\widehat{\sigma}ge^2 = 9.1$) that was 0.54 times higher than the genetic variance ($\widehat{\sigma}_g^2 = 5.9$). In the present study, the hybrid-year interaction variance was higher than the genetic variance in two of the WR-variable studied. Indeed, the hybrid-year variance ($\widehat{\sigma}_{gy}^2 = 0.935$) was 2.9 times higher ($\widehat{\sigma}_g^2 = 0.240$) than the genetic variance for DLGt, and 0.06 times higher ($\widehat{\sigma}_{gy}^2 = 0.018, \widehat{\sigma}_g^2 = 0.017$) than that for RDLGt. In contrast, it was 0.4 times lower ($\widehat{\sigma}_{gy}^2 = 0.006, \widehat{\sigma}_g^2 = 0.010$) than the genetic variance for RIPt.

In agreement with Fehr (1991), the hybrid-year interaction reflected the failure of hybrids to perform the same relative to each other across years. Previously we detected a genotype-isolate interaction of quantitative type, because the ranking of WRted genotypes was repeatable across *S. sclerotiorum* isolates (Castaño et al. 2001). However, in our trials, we did not evaluate the hybrid-isolate interaction. Thus, given that the host-pathogen interaction has low relative contribution to the total variance when horizontal resistances are considered (Parlevliet 1981), we assumed a negligible effect of isolates on the hybrid-year interaction. On the other hand, the lack of WR stability in hybrids due to their different ranking across years has been recently detected by Dinon (results not yet published). This author associated this instability with different meteorological effects during WR development. Thus, we assumed that the meteorological conditions could be associated with the hybrid-year interaction. However, further researches should be carried out to determine the impact of these meteorological variables.

The between-plots error variance, which represented the plot-to-plot variation across replications, had relatively low contribution, ranging from 8.5% (RIPt) to 4.5% (DLGt) (Table 2). Degener et al. (1999) estimated a plot-to-plot variance

($\hat{\sigma}^2 = 4.4$) that was 0.25 times lower than the genetic variance. Also, we found a similar trend for two of the WR variables evaluated. Indeed, the plot-to-plot error variance ($\hat{\sigma}^2 = 0.006$) was 0.4 times lower ($\hat{\sigma}_g^2 = 0.01$) than the genetic variance for RIPt, and 0.12 times lower ($\hat{\sigma}^2 = 0.015, \hat{\sigma}_g^2 = 0.017$) than that for RDLGt. In contrast, it was 1.15 times higher ($\hat{\sigma}^2 = 0.516, \hat{\sigma}_g^2 = 0.24$) than the genetic variance for DLGt.

The within-plot error variance, which represented the plant-to-plant variation within the plot, always showed the highest contribution, ranging from 57.7 (RIPt) to 70.7% (RDLGt) (Table 2). The within-plot error variance ($\hat{\sigma}_w^2 = 33.5$) estimated by Degener et al. (1999) was 4.7 times higher than the genetic variance. In the present study, we observed the same trend. Indeed, it was 3.1 times higher ($\hat{\sigma}_w^2 = 0.041, \hat{\sigma}_g^2 = 0.01$) for RIPt, 11.9 times higher ($\hat{\sigma}_w^2 = 0.219, \hat{\sigma}_g^2 = 0.017$) for RDLGt, and 31.9 times higher ($\hat{\sigma}_w^2 = 7.915, \hat{\sigma}_g^2 = 0.240$) for DLGt (Table 2).

According to Boomsma et al. (2010), plant-to-plant variability is consistently present in all field experiments, where the heterogeneity is expressed by differences among neighboring plants in the values of the variables determined (in our case, the WR variables). These differences may be originated by genetic and/or environmental causes (Fehr 1991). F1 hybrids are highly heterozygous but genetically homogeneous, whereas three-way hybrids are more heterogeneous than single ones. In our trials, all hybrids were single except four of them (11%) (BuckSurcoflor, Cauquén, Macon, MG60), which were three-way hybrids. Thus, it may be assumed that the contribution of genetic segregation to the within-plot variance was low. In agreement with Fehr (1991), environmental or non-genetic causes include variation in factors that, regardless of the genetic homogeneity observed in most hybrids (i.e., 89%), not all plants to be inoculated flowered the same day. Thus, inoculation and WR development occurred under different conditions of relative humidity and temperature. Given that the development and growth rate of *S. sclerotiorum* would have been faster in some inoculated capitula than in others, even within-plots, plant-to-plant heterogeneity could have been promoted. Although field observations were assessed by the same person (a fact that contributed to the homogeneity of the experimental design (Lucio and Sari 2017), WR severity was subjectively quantified. Besides, some capitula showed null lesion growths (i.e., DLG = 0) and this over-dispersion of data could also have affected the plant-to-plant variance. To reduce the inflated error variance and, consequently, the square mean error, we may propose that plants within-plots should be inoculated on the same date. This would allow all inoculated plants per plot to be under the same meteorological conditions and the error to be reduced. However, since a suitable number of plants would need to be at the same stage of development, plots should have higher number of plants and thus the experiment

will be larger and more expensive. Likewise, WR severity could also be objectively measured by photos taken on diseased capitula and quantified with special software. However, this will take longer times.

The highest DGD estimated for RIPt could be related to the lowest relative contribution of its non-controlled source of variation to the total variance, particularly its within-plot variance component (57.7%), but also to the highest relative contribution of its genetic variance (14.1%) respect to other ones (RDLGt = 5.5%, DLGt = 2.1%) (Table 2). However, in agreement with Fehr (1991), the latter contribution could be relatively diluted because genetic variance was a term used both in the numerator and denominator of the mathematical equation to estimate DGD.

The DGD value of DLGt (0.35) was 0.8 times higher than that of RDLGt (0.63) because it was relativized to the checks inoculated on the same date. This strategy was analogous to that suggested for RIP because, in agreement with Vear and Tourvieille (1988), the environmental variations related to different inoculation dates could be reduced. However, in the present study, the relative contributions of the within-plots (DGLt = 68.5%, RDGLt = 70.7%) and between-plots error (DGLt = 4.5%, RDGLt = 4.8%) to the total variance were not reduced. The effect of that relativization was more visible in the reduction of the contribution of the hybrid-year interaction variance (DLGt = 8.1%, RDLGt = 5.8%) and in the increase in genetic variances (DLGt = 2.1%, RDLGt = 5.5%) (Table 2).

The sunflower hybrids used in this work were from 19 different seed companies (INASE 2019) (Table 3). Most of them (53%) belonged to international companies producing and selling seeds in countries all around the world where sunflower is grown. Although the genetic formula of hybrids in Argentina is confidential (i.e., closed pedigree) (Castaño 2018), the diversity of seed companies used allows assuming that the genotypic variability of sunflower hybrids was well represented and that some of the genes controlling RIPt and RDLGt may be also present in hybrids commercialized outside Argentina, particularly in regions with risk of WR epiphytes.

The DGD value here estimated for RIPt was higher than that estimated by Godoy (2001) and the broad sense heritability (H^2) value estimated by Filippi et al. (2017) for RIP. The DGD is analogous to the H^2 (Jacquard, 1983) but, according to Fehr (1991), the DGD describes the ratio of genotypic to phenotypic variance among random genotypes when the reference population is cultivars of the species, as in our experiment, not a segregating population. In addition, the value estimated for RDLGt was also higher than that estimated previously for DLG (Castaño and Giussani 2006).

Field evaluations of quantitative resistances, as WR resistance, are the main bottleneck to obtain continuous progress in breeding for moderately resistant

cultivars (Willocquet et al. 2017). Our results showed that genotype-phenotype correlation for WR variables increased in relation to that mentioned in the bibliography. Thus, in breeding programs better genotypes for WR resistance could directly be selected with higher precision through their phenotypes in the field. Also, the detection of a higher number of quantitative resistance loci (QRLs) associated with WR resistance could be assured when, agreeing Dimitrijevic and Horn (2018), indirect selection is carried out through molecular markers.

Only six (16%) of the hybrids evaluated had a good level of resistance for RIPt and RDLGt because of their significant ($p < 0.05$) BLUP values (Table 3). Five showed the best abilities to extend the period of time necessary for ascospores to germinate, infect and develop enough mycelium for a disease symptom to be detected (i.e., RIPt), whereas only one showed the lowest mycelium progress of WR symptoms in its capitulum until maturity (i.e., RDLGt). These six hybrids would be of much interest in breeding programs as sources of resistance to develop moderately WR-resistant cultivars. Since they are commercial cultivars, their use would propitiate the generation of segregating populations with fewer non-desirable attributes than if less adapted germplasm were used (Fehr 1991).

Although the coefficient of relationship of Paraíso75, Paraíso27 and Paraíso65 may be high because common ancestors had to be used (Table 3), the probability that they have the same set of genes controlling RIPt as DM220AO and/or SPS3109 is quite low because they had to be derived from different germplasm (Falconer and Mackay 1996). Thus, these independent genetic sources could be used in breeding programs to increase the relative period without WR symptoms after *S. sclerotiorum* infections.

In agreement with Stear et al. (2012), the lack of statistical dependency among BLUPs suggested the absence of genetic correlation between RIPt and RDGLt and, consequently, the set of genes responsible for each variable are not shared, that is they are not co-inherited. A good example of this is the hybrid DM220AO, which showed the most favorable BLUP for RIPt (0.14) and the worst (0.18) for RDGLt (Table 3). Filippi et al. (2017) and Zubrzycki et al. (2017) estimated highly significant phenotypic correlations between several of the WR variables they evaluated. Besides, these authors suggested that these associated WR variables could be used as components of WR partial resistance. According to Parlevliet (1993), for a WR variable to be considered as a suitable component of WR partial resistance, it must show variability of responses, independence of other components, moderate heritability, and relatively low cost for its evaluation. Our results suggest that RIPt and RDLGt satisfied such requirements. Thus, the set of genes involved in RIPt and RDLGt can be useful to simultaneously select moderate WR resistance and high seed-yield sunflower hybrids (A. Giussani, not yet published).

In the present study, besides a greater relative contribution of the set of genes controlling RIP and RDLG to phenotypic variance, the genetic independence between them was revealed. So, the most accurate selection of favorable genotypes would facilitate the simultaneous accumulation of RIP and RDLG resistances by, for example, recurrent selection. Further researches must be oriented to evaluate the possibility to reduce allocated resources without resigning precision in the selection of moderately resistant hybrids. This may allow optimizing the time, personnel, experimental material and/or inoculum used in WR field evaluations.

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