1 Phenotyping sunflower genetic resources for response to Sclerotinia head rot: assessing variability for disease resistance breeding.

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23 **ABSTRACT**

24 Sclerotinia head rot (SHR) is one of the most serious constraints to sunflower (*Helianthus annuus* L. var.
25 *macrocarpus*) production worldwide. Here, we evaluated the response to SHR in a sunflower germplasm
26 collection, consisting of 137 inbred lines (ILs). Field trials were performed over five consecutive seasons
27 using a twice-replicated randomized complete-block design. Disease incidence, disease severity, incubation
28 period and area under disease progress curve for disease incidence and severity were determined after
29 controlled inoculation with the pathogen. Statistical analysis using mixed-effect models detected significant
30 differences among ILs for all variables (P<0.001). In addition, Principal Component Analysis (PCA) and
31 distance based methods were used to classify the ILs according to their response to SHR, with ILs ALB2/5261
32 and 5383 emerging as the most resistant. Broad-sense heritability estimates ranged from 20.64% for disease
33 severity to 10.58% for incubation period. The ample phenotypic variability of our collection, along with the
34 moderate heritability estimates, highlight the importance of molecular breeding approaches to gain new
35 insights into the genetic basis of sunflower resistance to SHR. The exhaustive phenotypic characterization
36 presented here provides a reliable set of variables to comprehensively evaluate the disease and identifies two
37 new sources of resistance to SHR.

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41 **INTRODUCTION**

42 Cultivated sunflower (*Helianthus annuus* L. var. macrocarpus) is one of the most important oilseed crops,
43 covering 25 million ha worldwide. The total annual production in the world is about 36 million metric tons,
44 mainly concentrated in the Russian Federation, Ukraine, European Union, and Argentina, which is the fourth
45 largest producer and the third oil exporter (www.sunflowernsa.com). However, there is a large gap between
46 the potential and actual yields, mainly due to biotic and abiotic constraints.

47 Sclerotinia head rot (SHR) is a major disease of sunflower, causing serious damage to production in almost
48 all the sunflower-growing areas of the world (Boland and Hall 1994). Achieving control of this disease is
49 challenging for three main reasons: *Sclerotinia sclerotiorum* (Lib.) de Bary, its etiological agent, is a
50 necrotrophic fungus able to survive for many years in the soil (Clarkson et al. 2013); it can infect a wide host
51 range, from annual plants to woody crops (Boland and Hall 1994); and effective therapeutic treatments are not
52 yet available (Van and Miller 2004). In this scenario, the development of resistant lines by pyramiding QTL
53 and candidate genes, using both classical and molecular breeding tools, appears to be one of the most
54 promising strategies for crop protection. Until recently, biparental mapping was the method of choice to
55 establish a link between phenotype and genotype and to identify the genomic regions underlying quantitative
56 variation of complex traits. Nowadays, interest is shifting to the use and mining of germplasm collections,
57 through association mapping approaches (Zhu and Salmeron 2007). Regardless of the breeding approach,
58 phenotyping of traits related to SHR resistance necessarily involves multi-environmental trials with a large
59 number of individuals.

60 A key aspect of screening for plant disease resistance is determining which are the most suitable variables and
61 methods for scoring the disease. In sunflower, SHR response has usually been evaluated using variables such
62 as disease severity (proportion of diseased host tissue), disease incidence (number or proportion of diseased
63 plants) and incubation period (number of days until onset of symptoms) (e.g. Bert et al. 2002, 2004; Castaño
64 and Giussani 2009; Vear 2004; Yue et al. 2008). Previous studies on SHR have reported different resistance
65 QTL depending on whether disease severity, disease incidence or incubation period were being considered,
66 suggesting that each of these variables capture different aspects of resistance (Yue et al. 2008).

67 From a methodological standpoint, a variety of methods have been employed for screening sunflower lines
68 and hybrids (Baldini et al. 2002; Castaño et al. 1993; Van and Miller 2004; Vear and Tourvieille de Labrouhe
69 1984, 1988). Among them, the application of ascospore suspensions on the floral surface is one of the most
70 commonly used testing procedures as it mimics the natural epidemiological cycle quite closely and allows
71 measurement of resistance throughout the whole pathogen's development on the host (Vear and Tourvieille de
72 Labrouhe 1988).

73 As with the above mentioned experimental procedures, the development of adequate statistical models
74 capable of providing accurate adjusted phenotypic means is also critical. Until now, the statistical analysis of
75 phenotypic data of sunflower resistance to SHR has mainly relied on analysis of variance (ANOVA) (Baldini
76 et al. 2002; Castaño et al. 1993; Mestries et al. 1998; Rönicke et al. 2005; Yue et al. 2008). However, the
77 classical ANOVA models, based on fixed effects and restricted to the assumptions of independence and
78 homoscedasticity of the error terms, cannot cope with either incomplete data sets or more complex scenarios
79 (e.g. incomplete blocks, heteroscedastic and/or correlated errors). Moreover, the classical models are also
80 inappropriate when working with non-normally distributed variables. In this context, estimation and hypotesis
81 testing based on extended and generalized linear mixed models emerge as the most suitable choice for the
82 analysis of SHR resistance data.

83 The Active Germplasm Bank of the Manfredi Experimental Station (AGB-IM)-Instituto Nacional de
84 Tecnología Agropecuaria (INTA) holds ca. 1200 accessions of cultivated sunflower, with representatives from
85 a broad range of geographic origins, and a large proportion of locally developed cultivars. This "Argentinean
86 germplasm" has a distinctive genetic constitution and is well adapted to the highly variable environmental
87 conditions of the sunflower cultivation areas of Argentina, i.e. from very hot in the semiarid region of the
88 Chaco Province to rather cold and humid in the southern Pampas (Filippi et al. 2015; Moreno et al. 2013).

89 In seeking to achieve a balance between genetic diversity and local adaptation, 137 inbred lines (ILs) from
90 the AGB-IM were selected to develop an association mapping population. This inbred line panel (ILP) is
91 currently used by the sunflower breeding program of INTA to detect useful genetic variation for a number of
92 agronomically important traits, including resistance to SHR (Fusari et al. 2012). Molecular diversity
93 assessment of this collection revealed the existence of three different genetic groups, with the

94 maintainer/restorer status being the most prevalent characteristic associated with group delimitation (Filippi et
95 al. 2015).
96 In this study, we used controlled inoculation and replicated field trials over five years to evaluate resistance to
97 SHR in the 137 ILP. The main goals of this work were to evaluate disease response using a comprehensive set
98 of variables and to identify new genetic sources of SHR resistance for sunflower breeding.

MATERIAL AND METHODS

Plant material and experimental field plot design

The sunflower ILP used for this study was composed of 137 ILs: 66 ILs from the sunflower breeding program of INTA for disease resistance, 54 ILs from the sunflower breeding program of INTA for abiotic stress tolerance and 17 public ILs of diverse genetic background. The complete list of ILs and their history in sunflower breeding programs is presented in supplementary Table S1. For more information about pedigree, country of origin and other agronomical characteristics see Filippi et al. (2015). All the inbred lines included in this study are available upon request from the AGB-IM.

Field trials (FTs) were conducted at Agricultural Experimental Station (AES) INTA Balcarce (37° 50’ 0’’ S, 58° 15’ 33’’ W, Province of Buenos Aires, Argentina) during growing seasons 2009/2010 (sowing date December 11th, 2009), 2010/2011 (sowing date December 6th, 2010), 2011/2012 (sowing date December 5th, 2011), 2012/1013 (sowing date December 6th, 2012) and 2013/2014 (sowing date December 9th, 2013). The first three and the last two FTs were performed under non-irrigation and irrigation regimes, respectively, with plants being spray-irrigated daily, at noon, for 20 min.

To break seed dormancy, seeds were incubated with a solution of gibberellic acid (GA^3) (100 ppm) for 60 min, followed by incubation at 10 °C until sowing. After treatment, seeds were planted by hand in Typic Argiudoll soil containing 5 % organic matter at pH 6.2.

The FTs were conducted in a randomized complete block design with two blocks. Each experimental unit was one row 9.0 m long by 0.7 m wide, with a planting distance of 0.25 m, resulting in 36 plants per row.

Fungal isolates

S. sclerotiorum sclerotia derived from naturally and experimentally infected plants were collected at AES INTA Balcarce (Buenos Aires Province, Argentina) every year and used for ascospore production according to Escande et al. (2002). Briefly, sclerotia were exposed to -18 °C ± 2 °C for 7 d and then cultivated in humid pasteurized Typic Argiudoll soil in darkness until germination, followed by incubation at 16 °C under continuous illumination of 2500 lux. Mature apothecia were collected in Petri dishes and incubated for 4 h to favor ascospore release. Ascospores were stored in plastic plates at -18 °C until inoculation. To produce

125 inoculum, the spores were washed from the plates with 10 mL sterile water, and adjusted to a concentration of
126 2500 ascospores/ mL with a Neubauer hemocytometer. Fresh inoculum was prepared at each inoculation date
127 immediately before use.

128 **Inoculation of inbred lines**

129 All plants were inoculated with the pathogen at the R5.2 flowering stage of the Schneiter and Miller's
130 scale (1981). Capitula were inoculated using a portable hand sprayer with 1 mL of inoculum (2500 ascospores
131 / mL) following the method of Tourvieille de Labrouhe and Vear (1984) with minor modifications (Escande et
132 al 2002) and immediately covered with paper bags up to 10 days post-inoculation (dpi). To check the efficacy
133 of the procedure, a susceptible cultivar was simultaneously inoculated with the tested ILs at all inoculation
134 dates.

135 Five phenotypic variables were registered: (a) Disease incidence (DI), i.e. the number of plants infected
136 over the number of plants inoculated in each row; (b) Disease severity (DS), i.e. average proportion of
137 capitulum rotted area of plants inoculated in each row; (c) the area under the disease progress curve for DI
138 (AUDPCI); (d) the area under the disease progress curve for DS (AUDPCS); and (e) incubation period (IP),
139 i.e. average number of days until onset of symptoms in each row. Evaluations were performed at 14, 17, 21,
140 24 and 28 dpi. Disease assessments were performed by a single person (CV Filippi) to avoid inter-rater error.

141 DI, DS, AUDPCI, AUDPCS and IP means per year and inoculation date were calculated and plotted
142 relative to global and annual means, respectively.

143 Mean, highest and lowest temperature (°C) and relative humidity (%) were registered daily by a weather
144 station located 400 m from the FT. Data can be accessed at
145 http://anterior.inta.gov.ar/balcarce/info/meteorologia/meteoro2.htm. The mean values of the variables
146 obtained at the different inoculation dates were correlated with temperature and relative humidity using
147 Spearman´s rank correlation.

148 **Statistical analysis**

149 Because of the different statistical properties of the phenotypic variables analyzed, appropriate linear or
150 generalized models were chosen accordingly. Models are described for each variable below. Random terms
151 are represented using at least one Latin letter.

152 *Disease incidence*

153 This variable measures the proportion of diseased plants relative to the total number of plants exposed. It
154 can be treated as a binomial count, with the following model being considered as suitable:

155 $$\text{Log}\left(\frac{\pi_{ijkl}}{1-\pi_{ijkl}}\right) = \mu + \lambda_i + c_j + f_{kj} + \lambda f_{ikj} + b_{lj} \quad (1)$$

156 where π_{ijkl} represents the probability of a plant becoming infected if it belongs to the inbred line *i*, evaluated
157 in field trial *j*, inoculated at date *k* in field trial *j*, and located in block *l* in field trial *j*. The terms $\lambda_i, c_j, f_{kj}, b_{lj}$,
158 λf_{ikj} refer to the effects of the inbred line *i*, field trial *j*, inoculation date *k* at field trial *j,* and block *l* within
159 trial *j*. Finally, λf_{ikj} refers to the interaction between IL and date of inoculation. Common assumptions for
160 random effects apply.

161 *Disease severity*

162 The DS was visually quantified, by applying a diagrammatic scale developed for SHR by the Plant
163 Pathology group of AES INTA Balcarce (ratio scale 0-100%, with 10 percent intervals). The generated data
164 were subjected to the square root-arcsine transformation before being fitted to a GLMM, with Y_{ijkl} being the
165 response variable defined as:

166 $$Y_{ijkl} = \mu + \lambda_i + c_j + f_{jk} + + \lambda f_{ikj} + b_{lj} + e_{ijkl} \quad (2)$$

167 where Y_{ijkl} represents the severity for the inbred line *i,* evaluated in field trial *j*, inoculated at date *k* in field
168 trial *j*, and located in block *l* in field trial *j.* All terms have the same meaning as in model (1), except for the
169 additional term e_{ijkl} that represents the classical normal error term.

170 *Area Under the Disease Progress Curve*

171 The estimation of the Area Under the Disease Progress Curve for DI and DS (AUDPCI and AUDPCS) was
172 carried out based on all data collected at 14, 17, 21, 24 and 28 dpi, using the formula described in Shaner and
173 Finney (1977):

$$\sum_{i=1}^{n}\left(\frac{x_{i+1} + x_i}{2}\right)(T_{i+1} - T_i)$$

174 where X_i is the proportion of diseased plants or proportion of capitulum rotted area at time T_i, $(T_{i+1} - T_i)$
175 represents the time (days) between two successive observations, and n is the total number of observations.
176 AUDPCI was estimated using the data from all five FTs, whereas AUDPCS was estimated using data from
177 the first four FTs, because of the lack of DS data for all days post inoculation (i.e. 14, 17, 21, 24 and 28 dpi)
178 for the 2013-2014 FT. After the normality assumption was verified, the adjusted means for these variables
179 were obtained by fitting to model as described above (2).

180 *Incubation period*

181 Adjusted incubation period means were obtained from a model as described above (2), after the normality
182 assumption was verified.

183 For all phenotypic variables, ILs´ means were subjected to multiple comparison tests using the DGC
184 procedure (Di Rienzo et al. 2002). Finally, the models described above were refitted, considering the IL effect
185 as random for estimating the contribution of the genotype to the phenotypic variance (i.e. broad-sense
186 heritability, H^2).

187 **Principal component and cluster analyses**

188 Principal Component Analysis (PCA) was carried out using the standardized adjusted means of all five
189 evaluated phenotypic variables.

190 Cluster assignment and selection of optimal number of clusters were done using the R package mclust
191 (Fraley and Raftery 2002, 2007). The model was assumed to be a mixture of diagonal, varying volume and
192 equal shape multivariate normal distributions (VEI model). In addition, a dendrogram was obtained from a
193 matrix of Euclidean distances calculated from the standardized phenotypic means using the unweighted pair-
194 group method (UPGMA).

195 All statistical analyses were conducted using InfoStat (Di Rienzo et al. 2014) and InfoGen (Balzarini and
196 Di Rienzo 2014).

197 **RESULTS**

198 We conducted a 5-year field experiment to evaluate the resistance of 137 sunflower ILs to *S. sclerotiorum*.
199 The overall mean of inoculated plants per experimental unit was 15. The number of ILs included in each FT
200 was variable: 69 in 2009/2010, 69 in 2010/2011, 112 in 2011/2012, and 137 in 2012/1013 and 2013/2014.

201 Controlled inoculation produced the typical disease symptoms. The lesions increased rapidly during the
202 first weeks of observation to reach a plateau at 28 dpi, and therefore we selected this time point as the final
203 stage of the disease.

204 The FTs showed differences in disease levels, regardless of the phenotypic variable under analysis. The
205 values of DI, DS and AUDPCI were above the overall mean in 2009/2010 and 2012/2013 and below it in
206 2011/2012 and 2013/2014, with values for 2010/2011 showing a different behavior depending on the variable
207 (Fig. 1 A, B, and C). AUDPCS showed the same pattern as DS, except for the 2009/2010 FT, in which the
208 AUDPCS was below the overall mean (Fig. 1B, and D). In addition, seasons with lower DI, DS and AUDPCI
209 had longer IP than those with higher values (Fig. 1E). DI and DS progress curves are presented in
210 supplementary Figs. S1 and S2.

211 Although all inoculation dates were suitable to produce disease, there were differences in the magnitude of
212 all variables among them, with means obtained at various inoculation dates showing large deviations from FT
213 means (Fig. 2).

214 Significant negative correlations were found between temperature on the inoculation date (maximum,
215 minimum and mean) and DI, DS and AUDPCI, while no correlation was observed with AUDPCS
216 (supplementary Table S2). In contrast, temperature was positively correlated with IP (Spearman´s rank
217 correlation test, $P<0.05$). No variable was correlated with relative humidity (supplementary Table S2).

218 **Statistical analysis of SHR-related phenotypic variables**

219 The adjusted means for DI, DS, AUDPCI, AUDPCS and IP were estimated by applying mixed-effect
220 models (1) and (2) (supplementary Table S3 A). The overall adjusted mean (and range) at 28 dpi was 0.55
221 (0.13-1) for DI, 0.58 (0.22-0.88) for DS, 5.86 (0.92-11.21) for AUDPCI, and 4.76 (0.14-8.99) for AUDPCS
222 (Fig. 3A, B, C, D, supplementary Table S4). The overall mean of IP was 18.92 dpi, ranging from 14.23 to

223 24.80 (Fig. 3E). Statistically significant differences were observed among ILs for all variables (*P* <0.001). The
224 DGC test (Di Rienzo et al. 2014) classified the ILs into two different groups according to DI, DS, AUDPCI,
225 AUDPCS and IP, respectively (supplementary Table S4).

226 Taken together, the results of the five variables suggest that the ILs ALB2/5261, 5383, 51084/5429 and 7-
227 1-1 (from the Sunflower Breeding Program of INTA) and the public IL RK416 are moderately resistant to
228 SHR. On the other hand, the ILs R459-4, B485-5, R463-3, R467-3, 5289, 5431 and B481-3 (from the
229 Sunflower Breeding Program of INTA) are highly susceptible to SHR. The phenotypic response of these ILs
230 was consistent across FTs, with the exception of ILs 5383 and 51084/5429 on the 2009/2010 FT, which
231 showed DI values above the FT mean (0.875 and 0.78 vs. 0.77), and RK416 on the 2012/2013 FT, which
232 showed DI and AUCPCI values slightly above the FT mean (0.825 vs. 0.76 and 8.24 vs 8.1, respectively).

233 Significant positive correlations were found between the adjusted means of DI, DS, AUDPCI and
234 AUDPCS, whereas IP was significantly negatively correlated with the other four variables (DI, DS, AUDPCI
235 and AUDPCS; Table 1).

236 The heritabilities of DI, DS, AUDPCI, AUDPCS and IP for each FT and across the FTs were estimated by
237 applying random effect models (supplementary Table S3, B). A broad distribution of H^2 values was obtained
238 across FTs, from high heritability estimates (e.g.: DI 2009/2010 FT, H^2 = 96.58 %,) to values near 0 (e.g.: DS
239 2011/2012 FT, H^2 = 0.45 %,) (supplementary Table S5). When all the FTs were considered together, the five
240 phenotypic variables under study showed moderate heritability, with DS having the highest (H^2 = 20.64 %)
241 and IP the lowest values (H^2 = 10.58 %) (supplementary Table S5).

242 **Principal component and cluster analyses**

243 A Principal Component Analysis (PCA) was carried out using the model-based adjusted means of the
244 phenotypic variables (Fig. 4). The first two axes explained 74.8 % and 14.0 % of the total variation,
245 respectively. All variables were positively correlated with PC 1, except for IP. AUDPCI showed the highest
246 correlation with this axis (0.94). All variables were positively correlated with PC 2, except for DS. DI showed
247 the highest correlation with this axis (0.59). The ILs are widely scattered throughout the PCA graph. The most
248 resistant ILs appear on the left of the upper-left quadrant of the PCA bi-plot, indicating lower DI, DS,

249 AUDPCI and AUDPCS and higher IP. Again, ALB2/5261 and 5383 responded better against SHR, followed
250 by 51084/5429. The VEI model in the mclust package split the 137 ILs into 6 groups. The assignment of ILs
251 to each group is given in supplementary Table S4. Means and standard deviations of all variables for each
252 group are provided in supplementary Table S6.

253 The Euclidean distances derived from model-based standardized means varied from 0.19 to 9.35, with an
254 average of 2.78. A dendrogram depicting the relationships among ILs is provided in supplementary Fig. S3.

255

256 **DISCUSSION**

257 Sunflower has extensive phenotypic and molecular diversity that can be exploited in breeding for
258 Sclerotinia head rot resistance. However, evaluation of SHR is a challenging task that involves the selection of
259 the inoculation method, disease descriptors and realistic statistical approaches for modeling the data.
260 Moreover, knowledge of the heritability of the trait is needed to aid in the decision-making process
261 for SHR resistance breeding.

262 Controlled inoculation is now recognized as the method of choice for disease assessment since the degree of
263 SHR infection is affected by weather conditions and the presence of sclerotia in the soil, two factors prone to
264 regional and temporal variation (Vear and Tourvieille de Labrouhe 1984). The ascospore method, developed
265 by Vear and Tourvieille de Labrouhe (1984), is one of the most employed testing procedures for screening
266 sunflower lines and hybrids, as it allows measurement of host resistance from the beginning of the infection
267 process. This method requires a careful determination of the optimal inoculum concentration since a high
268 inoculum pressure must be applied to ensure a sufficient number of infected plants. However, inoculum
269 concentrations higher than optimal will induce severe disease precluding differentiation among genotype
270 responses, while low inoculum concentrations will fail to produce symptoms in resistant genotypes. In the
271 present study, SHR phenotypic variables showed a wide range of variation indicating that the used inoculum
272 concentration was adequate to accurately measure disease.

273 Previous studies investigating the influence of sunflower genotypes and *S. sclerotiorum* isolates on SHR
274 response reported that both variables exerted highly significant effects, while they did not interact with each
275 other (Thuault and Tourvieille de Labrouhe 1988; Vear 2004). No changes in the resistance ranking of
276 sunflower genotypes were observed when testing *S. sclerotiorum* isolates with different levels of
277 aggressiveness. Thus, the authors concluded that breeding and disease resistance tests with any isolate or
278 population of *S. sclerotiorum* should be valid for all areas and many years (Vear 2004). These observations
279 suggest that sunflower has "horizontal" and "race non-specific" resistance to *S. sclerotiorum* and that the use
280 of a mixture of spores of different origins and natural populations is probably the safest way to ensure the
281 long-term stability of the results (Vear 2004). In the present work, the inoculation of capitula using a mixture
282 of ascospores derived from sclerotia found in naturally infected plants allowed a clear discrimination of

283 responses for the five phenotypic variables, i.e. disease incidence (DI), disease severity (DS), AUDPC
284 incidence (AUDPCI), AUDPC severity (AUDPCS) and incubation period (IP).

285 In our experiments, year-to-year differences in temperature on inoculation dates seem to have been
286 associated with disease occurrence. Indeed, temperature on inoculation date was significantly correlated with
287 four of the phenotypic variables evaluated (i.e. DI, DS, AUDPCI and IP), while the inoculation date effect
288 explained a considerable proportion of random variation in our GLMMs. The lowest infection levels occurred
289 in 2011/2012, which showed the highest temperatures (mean maximum and minimum temperatures of 35.6
290 and 23.3 °C, respectively). In contrast, the highest disease levels occurred during the 2009/2010 FT when
291 temperatures where the lowest (mean, maximum and minimum temperatures of 32 and 21.8 °C, respectively).
292 Taking into account the influence of temperature on the disease development, Tourvieille de Labrouhe and
293 Vear (1984) recommended that only materials inoculated on the same day should be used for comparisons
294 between different sunflower accessions. The protocol of ascospore inoculation requires capitula to be
295 inoculated at the R5.2 stage (Schneiter and Miller 1981), but the high variability in flowering time of
296 sunflower precludes the simultaneous treatment of a large number of ILs. In the past, this problem was
297 partially solved by expressing phenotypic measures relative to a susceptible control inoculated on the same
298 day (Bert et al. 2002, 2004; Castaño et al. 1993; Vear 2004). Under this approach, control individuals are
299 planted on staggered dates so that flowering controls are available throughout the flowering period. In
300 practice, however, this method is time and resource consuming. In this work, different GLMM models were
301 tested and applied to estimate the adjusted means of DI, DS, AUDPCI, AUDPCS and IP. Using the mixed
302 model approach, we were able to not only deal with complex data, but also to solve the problem of different
303 inoculation dates by including the random effect of the inoculation date in the statistical models. This
304 precluded the necessity of including controls to adjust the phenotypic measures and allowed us to test
305 genotype-by-environment interactions (GE, i.e. the differential genotypic response to different environments).

306 The DI is regarded as suitable for estimating resistance to fungal penetration, while the IP and the DS are
307 considered to be a measure of resistance to the spread of the fungus within the host tissues (Gentzbittel et al.
308 1998). DI is relatively objective and easy to obtain, making it suitable for scoring a large number of data,
309 particularly when these are collected by non-experts (Madden and Hughes 1995). In turn, DS is used to

310 characterize fungal diseases affecting specific plant tissues (Kranz 1988), as it most adequately describes the
311 spatio-temporal dynamics in terms of disease increase and spread (Madden and Campbell 1990). Besides the
312 final symptoms, the actual disease progression over time, as measured by AUDPCI and AUDPCS, is also
313 necessary for a more in-depth analysis of the disease. AUDPC has gained increasing importance for the
314 measurement of quantitative disease resistance in most foliar pathosystems (Jeger and Viljanen-Rollinson
315 2001) and it is currently applied to soil-borne diseases (Pouralibaba et al. 2015). Indeed, the analysis of
316 AUDPCI and AUDPCS, in combination with maximum DI and DS, enabled a more robust characterization of
317 the resistance response to rice blast (Long et al. 2000), tomato bacterial wilt (Rivard et al. 2012), apple brown
318 rot (Holb et al. 2012), and *Phytophthora* crown and root rot (Foster and Hausbeck, 2010).

319 In this study, individual and combined analyses of the five disease variables revealed that the ILs examined
320 here show a wide range of responses to SHR, with ALB2/5261 and 5383 appearing as the most resistant.
321 Moreover, we found significant positive correlations among DI, DS, AUDPCI and AUDPCs and negative
322 correlations between IP and the other four variables (DI, DS, AUDPCI and AUDPCS). The correlation
323 coefficients ranged from moderate to high but did not approach unity, indicating that the different variables
324 should still be considered as separate dimensions of the expression space of the disease. The correlation value
325 obtained between DI and DS ($r = 0.55$) was similar to that reported for sunflower by Yue et al. (2008) (r
326 between 0.57 and 0.64). The negative correlation between IP and DI, DS, AUDPCI and AUDPCS mentioned
327 above suggests that incubation time is shortened in years favorable for disease development, and vice versa. In
328 sunflower, similar correlation values were reported by Bert et al. (2002) (r between -0.293 and -0.477) and
329 Bert et al. (2004) ($r = 0.55$).

330 In addition to measuring correlations among disease variables, some authors have investigated the
331 relationship between SHR and morphological or field characters, as a proxy for disease resistance (Castaño et
332 al 1993, Hahn 2002). Hahn (2002) found that the physiological stage does not influence the results of head rot
333 tests. Although inbred lines showed significant differences for days of flowering, no correlations were
334 observed between this variable and resistance measures. These results support the proposals of Castaño et al.
335 (1993) that taking into account morphological and field characters will not improve the efficiency of breeding
336 programs for sunflower SHR.

337 Another key aspect in plant breeding is the determination of the trait's heritability. Broad-sense heritability 338 (H^2) expresses the extent to which individuals' phenotypes are determined by the genotypes (Falconer et al. 339 1996). In the breeding context, phenotypic selection would be efficient for high heritability traits, while 340 marker-assisted selection, via the biparental or the AM approach, appears as the best option for low 341 heritability traits. In this work, heritability was estimated by pooling data not only from the two replicates of 342 the five FTs, but also from the two replicates of each FT to obtain a single mean for each variable. When the 343 results of all FTs were taken together, the five phenotypic variables showed moderate heritability, with H^2 344 ranging between 20 % and 10.58 % (DS and IP, respectively). In turn, when each FT was analyzed separately, 345 H^2 showed a broader distribution, varying from high to very low heritability values. The broad distribution of 346 H^2 values resulting from the separate analysis of each FT is highly comparable to those obtained by previous 347 authors for DI, DS, IP and other SHR -related phenotypic variables (Bert et al. 2002, 2004; Mestries et al. 348 1998; Zubrzycki 2014).

349 Given that *S. sclerotiorum* is present in almost all sunflower growing regions of the world, one of the main 350 goals of SHR breeding is to ensure the long-term stability and broad usefulness of the desired characteristics. 351 In the present study, the phenotypic response of the moderately resistant ILs was consistent across FTs. 352 Moreover, sunflower public lines described as resistant by American and French research teams (e.g. 353 Maestries et al. 1998, Yue et al. 2008) behaved as resistant in our geographic area and with a local inoculum 354 challenge. In this context, it is expected that SHR results from evaluations performed at AES INTA Balcarce 355 will hold in different environments.

356 In comparison to previous screenings for SHR-resistance in sunflower (e.g. Castaño et al 2001, Hahn 2002, 357 Vear and Tourvieille de Labrouhe 1988), our phenotypic characterization relies on a larger set of ILs, new 358 disease variables, and more appropriate and realistic statistical approaches for modeling the data. Despite the 359 fact that no complete resistance was detected, our results reinforce the notion that different phenotypic 360 variables are required to fully capture disease response. In this context, the ILs ALB2/5261 and 5383 emerge 361 as the best candidates for breeding based on their lower DI, DS, AUDPCI and AUDPCS and higher IP values. 362 The ample phenotypic variability of our collection, along with the moderate heritability estimates, highlight

363 the importance of molecular breeding approaches to gain new insights into the genetic basis of sunflower

364 resistance to SHR.

365

366

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373

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468

TABLES

Table 1. Spearman's correlation analysis of Sclerotinia head rot-related variables. Coefficients (below diagonal) and *P*-values (above diagonal).

	DI	**DS**	**AUDPCI**	**AUDPCS**	**IP**
DI	1	<0.0001	<0.0001	<0.0001	<0.0001
DS	0.51	1	<0.0001	<0.0001	<0.0001
AUDPCI	0.9	0.72	1	<0.0001	<0.0001
AUDPCS	0.47	0.85	0.67	1	<0.0001
IP	-0.51	-0.77	-0.73	-0.71	1

DI: disease incidence; DS: disease severity; AUDPCI: area under the disease progress curve for DI; AUDPCS: area under the disease progress curve for DS; IP: incubation period.

475

476 **FIGURE CAPTIONS** [A1][A2]

477 **Figure 1.** Annual means of Sclerotinia head rot-related phenotypic variables relative to the overall mean. (A)
478 disease incidence; (B) disease severity; (C) area under the disease progress curve for disease incidence; (D)
479 area under the disease progress curve for disease severity; (E) incubation period. Horizontal lines represent the
480 overall mean.

481

482 **Figure 2.** Means of Sclerotinia head rot-related phenotypic variables per inoculation date relative to their
483 respective annual means. (A) disease incidence; (B) disease severity; (C) area under the disease progress
484 curve for disease incidence; (D) area under the disease progress curve for disease severity (data for the
485 2014/2015 FT is not available); (E) incubation period. Horizontal lines represent the annual means.

486

487 **Figure 3.** Frequency histograms of Sclerotinia head rot-related phenotypic variables (N=137).

488

489 **Figure 4.** PCA bi-plot based on the matrix of the adjusted means of the Sclerotinia head rot-related
490 phenotypic variables. Points represent inbred lines (ILs). Variables are indicated by lines extending from the
491 center of the graph. The ILs were colored based on their VEI group assignment. The most resistant ILs are
492 underlined.

493

494 **Supplementary Tables and Figures**

495 **Table S1**. Sunflower inbred lines included in this study.

496

497 **Table S2.** Correlation between Sclerotinia head rot-related phenotypic variables and meteorological
498 conditions along the dates of inoculation.

499

500 **Table S3.** Statistical analysis of the Sclerotinia head rot-related phenotypic variables examined in this study.

501

502 **Table S4.** Adjusted means of the Sclerotinia head rot-related phenotypic variables examined in this study.

503

504 **Table S5.** Summary statistics and heritability estimation for the Sclerotinia head rot-related phenotypic

505 variables examined in this study

506

507 **Table S6.** Means of Sclerotinia head rot-related phenotypic variables for the six groups identified by the

508 Principal Component Analysis using the VEI model in mclust.

509

510 **Figure S1.** Disease incidence progress curves. To facilitate visualization, a representative IL from each of the

511 six mclust groups was included in the graph. (A) 2009/2010 Field trial; (B) 2010/2011 Field trial; (C)

512 2011/2012 Field trial; (D) 2012/2013 Field trial; (E) 2013/2014 Field trial.

513

514 **Figure S2.** Disease severity progress curves. To facilitate visualization, a representative IL from each of the

515 six MCLUST groups was included in the graph. (A) 2009/2010 Field trial; (B) 2010/2011 Field trial; (C)

516 2011/2012 Field trial; (D) 2012/2013 Field trial.

517

518 **Figure S3.** Dendrogram based on Euclidean distances. The ILs were colored based on their VEI group

519 assignment.

520 [A3]