Precision in the genotype evaluation of sunflower white rot resistance since the amount of used resources

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ABSTRACT

- The resistance of sunflowers to white rot produced by *Sclerotinia sclerotiorum* infections on capitula is of horizontal type. We know the magnitude and expression of this disease resistance are highly affected by environmental conditions. Thus to obtain an adequate precision when the level of white rot is evaluated, several replicated experiments having many plants by plot have to be carried out. It implies however the use of a lot of resources. Therefore, the aim of this study was to determine the optimal allocation of resources that is the amount of plots and plants/plot, in experimental designs for evaluating sunflower white rot resistance.

- During 2009 and 2010, two experiments were made in Balcarce. In both, 37 sunflower commercial hybrids were grown following a randomized complete block design, with 3 replications. At each plot, 12 plants were randomly selected and then infected following the French protocol with an aqueous suspension of *S. sclerotiorum* ascospores. Inoculated capitula were monitored twice a week until the white rot symptoms appeared, then afterwards once a week till crop maturity. The relative incubation period (RIP) of white rot and the daily growth lesion rate (DGL), were measured. Data were analyzed under a random effects model which included the following effects: year (environment), replications within environment, genotype, genotype by environment interaction (GE interaction) and two sources of error: between and within plots. The variance components were estimated by restricted maximum likelihood and were used to calculate the degree of genetic determination (DGD) by Holland's generalization for unbalanced data, because some plots had different numbers of diseased capitula. To evaluate the optimal allocation of resources, random samples of plants were taken from each plot for different combinations of sample sizes and numbers of replications. For each subset of data the same model was fitted and the DGD estimated. Changes in DGD values and the lost of precision were both evaluated in terms of number of plants/plot and number of replications.

- The GE interaction variance components were significant for both variables ($p_{RIP} < 0.0001$, $p_{DGL} = 0.0078$). Generally when the number of used resources decreases, the estimated DGD value also decreases. However for lower values of used resources, DGD variability increases. When all available resources (i.e. 12 plants/plot and 3 replications) were used, RIP's DGD value was 0.651. If 8 plants/plot and 3 replications were considered the average value of DGD decreases to 0.649. So the DGD value was underestimated by 0.3% but 33.3% of resources saved. For 12 plants/plot and two replications (33.3% of resources saved), the DGD mean value was 0.622 and the underestimation reached a mean value of 4.5%. For the DGL the estimated DGD value was 0.630 when all resources were used, and it dropped to 0.610 using 8 plants/plot in 3 replications. The mean underestimation was 3.2%. If 12 plants/plot in two replications are considered the DGD mean value dropped to 0.545, an underestimation of 13.5%. Accuracy and precision of estimated DGD values dropped when the same quantity of resources were allocated in two instead of three replications. When 8 plants/plot were allocated in three blocks, underestimating RIP's DGD value more than 5% had a probability of 0.12. When the same amount of resources was allocated in two blocks, the probability was 0.33. For DGL, using 8 plants/plot and three replications, the probability of underestimating the DGD value more than 5% was 0.37 and increased to 0.56 when 12 plants/plot and two replications were used.

- The recommendation of the optimal resources allocation in sunflower breeding programs, for white rot resistance, could change when the results from experiments that are been carry out in more environments get into account.

- The actual results imply that the optimal allocation of resources to evaluate the level of resistance to white rot is 8 plants/plot and three replications, since the lost of accuracy was quite low in relation to 12 plants/plot in three blocks.

Key words: allocation of resources, breeding, capitula, resistance, *Sclerotinia sclerotiorum*.

INTRODUCTION

In sunflower (*Helianthus annuus* L), the white rot on capitula (WRC) produced by *Sclerotinia sclerotiorum* is a disease that may devastate this oleaginous crop if environmental conditions are favorable (Masirevic and Gulya, 1992). The southeast of Buenos Aires Province is the main sunflower production area in Argentina. The level of incidence of the WRC is one of the most important factors determining sunflower yield (Sadras et al., 2000).

The resistance of WRC is of horizontal type (Castaño et al., 2001) and its expression is highly dependent on the environmental conditions. Castaño and Giussani (2009) proposed that WRC may be considered as a disease composed by different phases of a cycle. Thus, the evaluation of WRC can be done through certain variables quantifying the partial resistance. The incubation period is a quantitative variable that measures the time in days in which the first visible symptoms are manifested because the pathogen penetrated into the capitulum and its mycelium colonized the parenchyma tissue of the inflorescence. The Relative Incubation Period (RIP) is the incubation period of genotypes in relation to that one of the check. The Daily Growth Lesion rate (DGL) is another quantitative disease variable measuring the daily rate of the disease severity increase until the maximum level of disease severity is reached.

Sunflower breeding programs develop cultivars with moderate levels of WRC resistance (Van Becelaere and Miller, 2004). Previous reports showed a weak relationship between the phenotype and genotype, in of some components of partial resistance (Godoy et al., 2005). In order to accurately estimate the degree of genetic determination (DGD) of these components, several field trials including many replications across environments must be carried out.

Insufficient research had be done to find the best combination of resources (i.e. number of environments, replications and plants evaluated per plot) to optimize their allocation for the evaluation of WRC. To our knowledge, only one previous report made by Degener et al. (1999) in Germany has discussed the optimal allocation of resources in evaluating sunflower genotypes for resistance to *Sclerotinia*. They found that an experiment carried out in four environments with two replications and between five and six evaluated plants per plot was the best combination of resources to optimize the selection response to stem rot resistance. Similar information related to WRC was nevertheless not found. Therefore, the aim of this study is to determine the optimal allocation of plants per plot and number of replications for the evaluation of WRC resistance in sunflower.

MATERIALS AND METHODS

During 2009 and 2010, two experiments were made in Balcarce, Argentina. In both, 37 sunflower commercial hybrids were grown following a randomized complete block design, with 3 replications. At each plot, 12 plants were randomly selected and then infected following the French protocol (Vear and Tourvieille, 1984) at the R5.3 stage (Schneiter and Miller, 1981) or its analogous F3.2 (Cetiom, 1992) with 5 ml of an aqueous suspension containing 5000 ascospores of S. sclerotiorum/ml.

After inoculation, capitula were covered with Kraft paper bags until crop maturity. There was irrigation by sprinklers. From 15-20 days after infection, all inoculated capitula were monitored twice a week until the white rot symptoms appeared and then once a week till crop maturity. Two components of partial resistance were assessed: the relative incubation period (RIP) of white rot, that is the relative delay (in days) from inoculation to symptom appearance relative to controls, and the daily growth lesion rate (DGL), which estimates the progress of white rot until the maximum value of disease severity is reached, were measured.

Data were analyzed under a random effect model which included the following effects: year (environment), replications within environment, genotype, genotype by environment interaction (GE interaction) and two sources of error: between and within plots.

Since the model residuals did not fit to a normal distribution, Box and Cox method (Box and Cox, 1964) was used to find an adequate transformation, data were then transformed by the potency functions $(RIP)^{-0,5}$ and $(DGL)^{0,25}$. The variance components were estimated by restricted maximum likelihood (Patterson and Thompson, 1971) and they were used to calculate the degree of genetic determination (DGD). For that, the Holland method (Holland et al, 2003) for unbalanced data was utilized, because in some plots the disease incidence was not 100%.

The DGD was estimated as follows:

$$DGD = \dfrac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \dfrac{\hat{\sigma}_{ga}^2}{a} + \dfrac{\hat{\sigma}^2}{an} + \dfrac{\hat{\sigma}_s^2}{ans}}$$

DGD: degree of genetic determination, $\hat{\sigma}_g^2$ genetic variance, $\hat{\sigma}_{ga}^2$ genotype x environment interaction variance, $\hat{\sigma}^2$ error variance, $\hat{\sigma}_s^2$ within error variance, a: years, n: replications, s: harmonic mean of the observed plants per plot.

For each reduced unit of plants/plot and replications, we took 360 random samples in which the DGD was estimated, excepting that one comprised by 12 plants/plot and 2 replications because only 9 different samples could be extracted.

The relationship between the DGD and the amount of evaluated resources by genotype (NRC) was estimated. Its value allows detecting the optimal allocation of resources from which the DGD of components of white rot partial resistance could be accurately estimated. All calculations were performed using the software R program R (R Development Core Team, 2011) and the components of variance components were estimated with the lme4 package (Bates et al., 2011).

RESULTS

Table 1 shows the mean values and standard deviations of the analyzed variables. A high average value of white rot incidence across to both experiments (95.85%) was estimated. This suggests the appearance of a high relative number of rotted capitula from which the RIP (1.02) and DGL (6.1%/day) mean values were estimated

Table 1. Mean values and standard deviations for white rot incidence (INC), relative incubation period (RIP) and daily growth lesion rate (DGL)

Statistics	Variables		
	INC	RIP	DGL (%/day)
Mean	95.85	1.02	6.10
Standard deviation	10.69	0.23	3.23

Analysis of variance detected significant ($p<0.01$) genotype x environment interaction, for both variables under study.

For both variables, we considered the more reliable estimate of DGD the one calculated with all the resources allocated since it was obtained with the maximum amount of data. While sometimes the DGD calculated with fewer resources was greater than the one obtained with all the data available, generally underestimated it. Furthermore, DGD underestimation and variability growth when the amount of resources decreased (Fig 1 and 2).

Figure 1 shows the variation of DGD estimated values across to the allocated resources when RIP was evaluated.
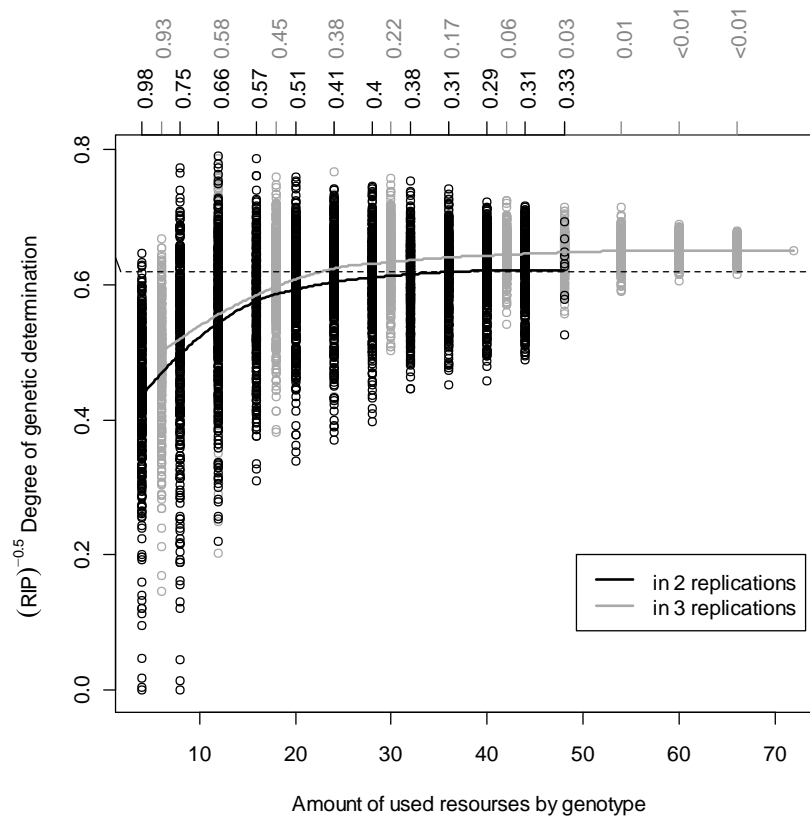
Fig 1. Estimated DGD of RIP based on the amount of allocated resources per genotype. The black dotted line indicates an underestimation of 5% of the most reliable DGD value. The solid lines mark a smoothed trend of DGD. Upper axis shows the probability of underestimating in more than 5% the DGD value using all the resources.

DGD value of RIP reached 0.651 when all available resources (i.e. 12 plants/plot and 3 replications, NRC=72) were used. However, if we consider only 8 plants/plot and 3 replications (NRC =48) the average value of DGD decreases to 0.649. So the DGD value was underestimated by 0.3% but 33.3% of resources saved.

Given the same amount of saved resources (i.e. 33%) but arranging them in 2 replications and 12 plants/plot, the DGD mean value decreased 0.622 and the underestimation reached a mean value of 4.5%. The empirical distribution of data showed that the loss of accuracy is higher when we used 2 rather than 3 replications.

If 8 plants/plot and 3 replications were used, the probability of underestimating the PIR value more than 5% is 0.12, and increased to 0.33 when 12 plants/plot and 2 replications are considered.

Moreover, if only 4 plants/plot/cultivar were evaluated the accuracy of the estimated DGL is very low the range of DGD values is zero to the value calculated with all data. The probability of underestimating the DGD value more than 5% would be of 0.98.

Figure 2 shows the variation of DGD estimated values across to the amount of allocated resources when DGL was evaluated. The DGD estimated values for the DGL show higher variability than for the RIP (Fig. 2). The graphic shows that the DGD estimated values decreases as higher amount of resources are used. However, these DGD values seemed to be underestimated.
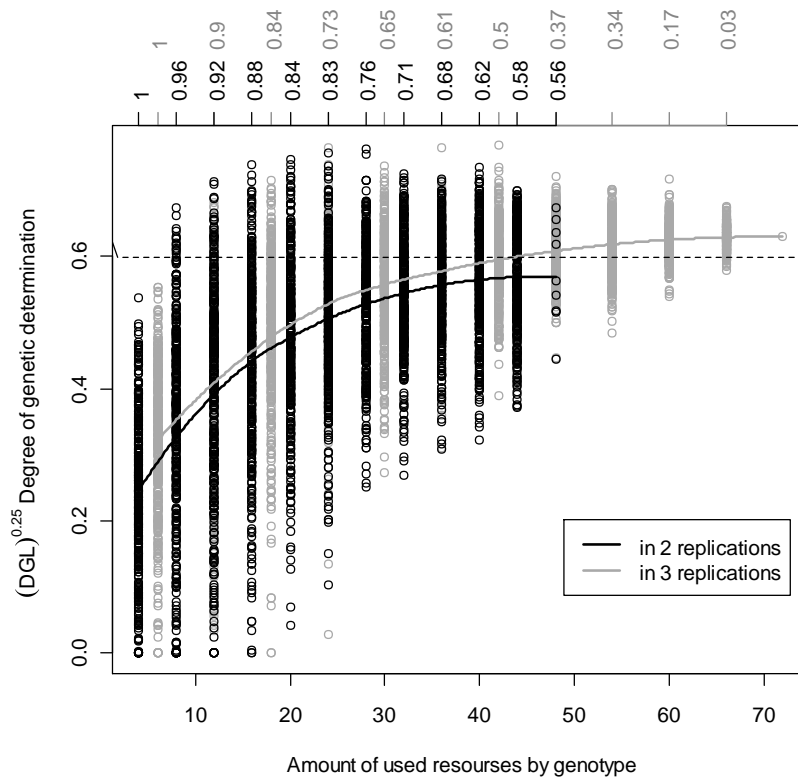
Fig. 2 Estimated DGD of DGL based on the amount of allocated resources per genotype. The black dotted line indicates an underestimation of 5% of the most reliable DGD value. The solid lines mark a smoothed trend of DGD. Upper axis shows the probability of underestimating in more than 5% the DGD value using all the resources.

When the whole of resources were used the DGD estimated value of DGL was 0.630, but it dropped to 0.610 for 8 plants/plot and 3 replications (NRC =48). The mean of underestimation was 3.2%. If the same amount of resources were allocated in 12 plants/plot and 2 replications the DGD mean value dropped to 0.545. Thus, there would be an underestimation of 13.5%.

The empirical distribution of data for 8 plants/plot and 3 replications showed that the probability of underestimating the DGD value more than 5% would be of 0.37. This probability increases to 0.56 when 12 plants/plot and 2 replications are considered. The DGL was the variable whose DGD values are less accurately estimated when low resources are allocated.

As Figure 2 shows, if 4 or 6 plants were assessed by cultivar, the probability of underestimating the DGD value more than 5% would be 1 and the most reliable DGD value would not be placed among of all possible estimated values.

DISCUSSION

In this work the data were from only two years. Therefore, it is considered that the estimated IGE variance may not be the most adequate. However, the IGE effect was significant for both white rot variables and this agrees with that found by Godoy et al. (2005). Then the white rot resistance in sunflower is affected by the environmental effects.

The underestimation of DGD values would not be severe when we work with a fixed number of plants/cultivar over 2 or 3 replications. This agrees the conclusion of Degener et al. (1999), but they studied the effect of diminishing the amount of allocated resources in the estimation of DGD value without re estimating the variance components as we did. In fact, they used the components of variance estimated with all obtained data and they did not measure the loss of precision when these components are estimated with a smaller number of evaluated plants.

In order to improve the efficiency of allocated resources and given a fixed number of plants/cultivar to be evaluated in two experiments, the empirical distribution of current data showed a clear decrease in the precision in the calculation of the DGD value when sunflower plants were distributed in 2 instead of 3

replications. This is due to the large variability in the estimates of DGD when the experimental design has one replication less. This result may have an immediately impact on a sunflower breeding program when the efficiency of allocated resources want to be improved.

In a previous work, Castaño et al. (1993) discussed the necessity to evaluate between 10 to 26 plants/genotype to have a reasonable accuracy in the estimation of the relative incubation period. Our results showed that the probability of underestimating the DGD value more than 5% is 0.38 (Fig. 1) if 12 plants/genotype/year distributed in 3 replications are considered. Also, if the goal of the sunflower breeder is to select genotypes with a good DGL behavior using only 3 plants/plot, 3 replications and 2 environments (i.e. 9 plants/genotype/year), the risk of underestimating the DGD value would be quite large (Fig. 2) and a certain probability that this value is zero. If this happens sunflower breeder could erroneously conclude that the observed variability in the field was due only to the environmental component and none of the cultivars stand out from others.

From our results, for a risk of under estimating RIP's DGD lower than 10%, the minimum amount of resources needed per cultivar per year is 7 plants/plot and 3 replications, whereas for DGL's DGD this minimum rise to 11 plants/plot and 3 replications.

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