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EFFECTIVENESS OF COMPONENTS OF PARTIAL RESISTANCE IN ASSESSING WHITE ROT OF SUNFLOWER HEAD

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SUMMARY

White rot is an important disease of sunflowers. Sunflower genotypic responses to S. *sclerotiorum* have to be evaluated with precision. White rot disease could be assessed by components of partial resistance and this would help to evaluate accurately genotypic responses. Here we wish to show which of variables or components of white rot are most suitable for describing sunflower performance during disease cycle. Twelve sunflower genotypes have been grown in an experiment and then inoculated. Disease incidence, relative incubation period, disease severity (observed at two moments), and the growth lesion rate were scored. All variables showed variability among the genotypes. The disease incidence and relative incubation period were confirmed as components of partial resistance. The results also indicated that the growth lesion rate during the intermediate and last phases of the disease seemed most appropriate to be used simultaneously with disease incidence and relative incubation period. If these three components were selected simultaneously, developed cultivars would have a more complex resistance and they would be less prone to the disease.

Key words: breeding for resistance, cultivars, selection efficiency, *Sclerotinia sclerotiorum*

INTRODUCTION

Sclerotinia sclerotiorum is a common and widespread fungus infecting sunflower roots, stems, buds and head (Gulya *et al.*, 1997). In the Argentinean sunflower growing areas as well as in some European countries, USA and Japan, the

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most important attack by the pathogen is on head when it is referred to as the white rot disease.

The use of moderately resistant cultivars by farmers could be considered as one of the best control methods. Sowing these hybrids must however be considered in a framework of an integrated control system which decreases the risk of the occurrence of parasitic populations resistant to chemicals, hyperparasites and resistance genes (Castaño *et al.*, 1991).

The white rot resistance in sunflowers is of horizontal type (Castaño *et al.*, 2001a). The resistance is considered like a quantitative character because it implies several minor genes clustered in QRL's (quantitative resistance loci) as well as a low heritability, because of the high environmental effects modifying both the magnitude and expression of symptoms. Therefore, the description of genotypic performance of white rotted sunflower is not a simple task and can consequently cause a slow genetic progress by selection.

In Argentina, sunflower breeders evaluate the white rot performance assessing only disease incidence (DI) because they are not interested of knowing if sunflower inflorescences have either high or low lesions nor if symptoms appeared just after the beginning of flowering or afterwards, close to crop maturity. In this situation, genotypes are selected only because they have a low number of diseased plants. This strategy could explain the low genetic progress in white rot resistance development when new cultivars, released in the local market during the period 1980-99, were considered (Odriozola *et al.*, 2006).

White rot was described as a disease composed by different phases, the whole process beginning at flowering (*i.e.*, infection) and ending at maturity (Castaño, 2007). Therefore, a key to improving the development of new sunflower cultivars with adequate level of white rot resistance would have to include an evaluation of the genotypic performance during the different steps of the disease. Presently in Argentina, the penetration resistance into the inflorescence is the only parameter evaluated (Odriozola *et al.*, 2006), and it corresponds barely to evaluating the performance of genotypes at the first stage of the disease. The strategy carried out by Argentinean breeders is evidently incomplete since the intermediate and final stages of the disease development are not evaluated. Thus, it becomes necessary to complete the genotypic description measuring some additional components of resistance to white rot.

Components of partial resistance were utilized in the following host-pathogen interactions: barley-*Puccinia graminis hordei*, potato-*Phytophtora infestans*, and rice-*Xantomonas campestris oryzae* (Parlevliet, 1993). To be selected for, components showing variability of responses as well as significant correlations with disease resistance must also provide lower costs of disease evaluation and higher heritability of field resistance.

In sunflower, the DI and the relative incubation period (RIP), also called incubation index or latency index (Vear and Guillaumin, 1977; Tourvieille and Vear, 1984;

Vear and Tourvieille, 1987), are components of partial resistance to white rot (Castaño *et al.*, 2001a,b). Using both variables Godoy *et al.* (2005) detected a series of sunflower hybrids with adequate adaptation to be grown in Argentinean sites with epiphyte risk. The use of the second white rot variable (=RIP) in the hybrid selection by white rot resistance have to be considered as a progress given that the selected genotypes will have not only lower number of diseased plants than the susceptible ones, but also a delayed occurrence of first white rot symptoms.

The latter component could still be considered as an incomplete strategy because the final phases of the white rot disease are not even evaluated. Disease severity (SEV, Russi *et al.*, 2004), which takes into account the white rot area of the head, and daily growth lesion rate, (DGL, Castaño and Giussani, 2006), which estimates white rot progress until maximum disease severity is reached, help to estimate the genotype performance in this period of the white rot disease.

So we have four variables describing the merits of sunflower against *Sclerotinia* on the head: DI, RIP, SEV, and DGL. If these four variables are evaluated at sequentially, the entire cycle of white rot would be assessed. But could all these four variables be used together? Giussani *et al.* (2008b) state that breeders should know that the number of objectives during the selection process (*i.e.*, characters to be selected) has to be as low as possible, in order to increase the probability of finding individuals having all desired attributes in the population selected (Fehr, 1987).

This article shows the results obtained in one year, when five variables were measured simultaneously after *Sclerotinia* infection on the head in an attempt to determine the most suitable variable to be used at different phases of white rot development in sunflowers.

MATERIALS AND METHODS

Twelve sunflower genotypes evaluated previously by Giussani *et al.* (2008a) for white rot resistance, of which six were commercial hybrids and six inbred lines, were grown at Balcarce, Argentina, following a randomized complete block design with two replicates. Experimental units consisted of a row 5 m long and 0.70 m wide, containing at least 15 plants. An old sunflower hybrid having a long white rot incubation period was also utilized as resistance check and sown at four different dates, at one-week intervals, to assure an extended period of flowering.

Sclerotinia sclerotiorum ascospores needed for inoculum were obtained following the procedure of Castaño and Rodríguez (1987). All sunflower heads were inoculated when the three external rows of hermaphrodite disk flowers at each inflorescence were at the pistillate stage: R5.3 (Schneiter and Miller, 1981) or its homologous stage: F3.2 (Cetiom, 1992). The floral surface of each head received an aqueous suspension containing around 25000 ascospores. Inoculations were made twice a week, each plant being infected once. At each infection date, 15 plants of the resistance check were also infected. Heads were covered immediately after inoculation with kraft paper bags in order to assure the infection. A commercial overhead sprinkler irrigation system was used until the end of the experiment at crop maturity.

Starting 20 days after inoculation, each head was observed at least once a week until crop maturity and the date of appearance of white rot symptoms recorded. The areas of the rotted head and the surface of the inflorescence side were also scored. At crop maturity (M4, Cetiom, 1992), disease incidence (DI) by plot was calculated as: [number of heads showing symptoms/number of inoculated heads]*100. The relative incubation period (RIP) was estimated as the plot mean value of the incubation index (*e.g.*, incubation period of genotype/average of incubation period of the resistance check inoculated at the same date) of each inoculated head. The area of each rotted head was valued as disease severity and this was estimated 40 days after inoculation (SEV-40dai) and when the severity reached its maximum value (SEV-Mx). Finally, the daily growth of the rotted area (DGR), from the date of first symptoms occurrence until the date the maximum severity was reached, was estimated as follows. For each diseased head, the speed of lesion development (in %/day) was calculated by the regression coefficient of the disease severity on the date at which white rot symptoms were observed. Plot means of SEV-40dai, SEV-Mx, and DGR were then estimated.

Variability of genotype performances and the relationship between *Sclerotinia* responses were afterwards analyzed according to Reza-Hoshmand (1998).

RESULTS

The values of general means, differences between maximum and minimum values, and coefficients of variability, obtained from the 12 sunflower genotypes inoculated with *S. sclerotiorum* are presented in Table 1.

Table 1: Descriptive statistic measures of five variables obtained from *Sclerotinia sclerotiorum* inoculation of sunflower heads

White rot variable	General mean	Range	Coefficient of variability (%)
Disease incidence (%)\ DI	99	93-100	1
Relative incubation period\ RIP	0.8	0.5-0.9	9
Disease severity at 40 dai (%)\ SEV-40dai	93	73-100	2
Maximum disease severity (%)\ SEV-Mx	98	90-100	3
Daily growth lesion rate (%)\ DGR	6.4	4.4-8.2	10

The average DI was 99%. Most of the evaluated genotypes (83%) showed white rot symptoms on the head. The range value oscillated by 7%. The coefficient of variability of 1% suggests a high precision of data and an almost null non-controlled variation. Although the general mean was high, the analysis of variance showed significant differences ($\alpha=0.003$) among the genotypes.

The mean of RIP was lower than one (0.8) and this indicates that, on average, first symptoms were detected earlier in the 12 evaluated genotypes than in the resistance check. The range of symptoms was 0.4 and the coefficient of variation reached CV=9%. There were significant differences (α=0.002) in the period in which first disease symptoms occurred.

The SEV-40 dai mean value reached 93%; therefore, a high proportion of rotted heads was achieved at the physiological maturity stage. The head area with symptoms fluctuated between 73 and 100%, and the coefficient of variability was CV=2%. The analysis of variance showed that the genotypes differed (α=0.000) in SEV-40 dai.

The SEV-Mx mean value was 98%. At maturity, the genotypes reached a very high proportion of rotted area in heads. Disease symptoms ranged by 10% and the coefficient of variability was CV=3%. Despite the high SEV-Mx average, the analysis of variance could still detect significant differences (α=0.048) among the genotypes' responses.

The average DGR reached 6.4%. The symptoms ranged by 3.8% and the coefficient of variability was CV=10%. The genotypes showed significant (α=0.009) responses in the analysis of variance.

In order to establish the degree of association between the variables describing the merits of genotypes in different phases of the white rot development process, simple correlation coefficients were calculated (Table 2).

Table 2: Linear and Spearman (in italics) correlation coefficients among the five variables obtained for *Sclerotinia* inoculation of sunflower heads

	RIP	SEV-40dai	SEV-Mx	DGR
DI	-0.05	*0.43*	*0.50*	*0.10*
RIP		-0.52*	-0.64*	-0.19
SEV-40dai			*0.68**	*0.59**
SEV-Mx				*0.19*

*significant at α<0.05

Of the 10 calculated coefficients, only four of them were different (α<0.05) from zero (RIP-SEV-40dai, RIP-SEV-Mx, SEV-40dai-SEV-Mx, SEV-40dai-DGR). There was no significant relationship when the DI was involved. Therefore, the relative number of plants having rotted heads was independent of the other quantified variables.

The significant and negative associations between RIP on one side and SEV-40dai and SEV-Mx on the other (r=-0.52 and r=-0.64, respectively) indicated the nature of the inverse relationship between them. The genotypes that were late in showing first symptoms generally had a low disease severity.

Concerning the SEV-40 dai, the two significant and positive Spearman correlation values suggest that the relative size of white rot on the head were directly related with SEV-Mx (r_S=0.68) and DGR (r_S=0.59). The genotypes having large rotted area of the head at the physiological maturity stage generally reached fast the maximum disease severity.

DISCUSSION

White rot data were obtained only from a field experiment. Therefore, it is necessary to conduct further experiments involving additional sunflower germplasm as well as additional sites to evaluate the reproducibility of the results. However, if we consider that this is the first time that components of partial resistance for white rot are used in sunflowers, these results should be of interest to be applied in the characterization of genotypes in plant breeding programs for white rot resistance.

The analysis of variance detected significant differences among the genotypes for all variables describing the different phases of white rot development. High average values for DI, SEV-40 dai and SEV-Mx were calculated (Table 1). This could be associated to the protocol of inoculation where a high inoculum pressure as well as paper bags covering sunflower heads until the end of the experiment were used. Because of the necessity to have a minimum number of diseased plants from which the RIP and DGR variables are estimated (Castaño *et al.*, 1993; Godoy *et al.*, 2005), the use of this methodology for inoculating sunflower heads is justified.

The correlation analyses (Table 2) showed that DI and RIP were not related. Independence between the variables was also shown by Castaño *et al.* (1993, 2001a, 2001b). The level of resistance to the penetration of *Sclerotinia sclerotiorum* shown by the sunflower inflorescence does not depend, therefore, of the degree of resistance of sunflower against the colonization and growth of the pathogen mycelium during the early stages of the disease development.

In order to complete the required genotypic characterization, the next step to be made is determining which of the SEV-40dai, SEV-Mx and DGR could be used simultaneously with DI and RIP. To select useful variables, we need to establish relationships among them. First, the correlation value between both disease severities (Table 2), SEV-40dai and SEV-Mx, was relatively high ($r_S=0.68$) and significantly ($\alpha<0.05$) different from zero. This coefficient indicates that SEV-40dai conditioned the SEV-Mx.

In the practice, the protocol used for evaluating the disease severity is analogous to the mycelium test, an old resistance test proposed by Vear and Guillaumin (1977). This mycelium test, that must be carried out in growth chambers, estimates the resistance of sunflower epidermal tissues to the mycelium colonization on the head and additionally measures the resistance to pathogen penetration into the inflorescence (Castaño *et al.*, 1993). In our work, the disease severities were independent of DI. Therefore, if disease severity is considered as component of partial resistance, this could be considered as an advantage because white rot response may be scored under field conditions, not under controlled ones.

In the present work, the occurrence of significant relationships between the disease severities estimated at two moments nevertheless discouraged their simultaneous use because, according to Parlevliet (1993), components of partial resistance could be related but their degrees of association should be as low as possible. So, the probability of finding common resistance genes, controlling the white rot severity at the physiological maturity stage of sunflower development, is diminished. Therefore, it seems necessary choosing only one of SEV-40dai and SEV-Mx to reduce the number of practices in the field as well as to increase the selection efficiency for white rot performance. In this sense, the strategy to be carried out could be related to knowing the strengths and weaknesses of each variable.

While the non-controlled variation for disease severity, measured at two moments, was low in both cases, descriptive statistics (Table 1) showed that the coefficient of variability was lower for SEV-40dai (CV=2%) than for SEV-Mx (CV=3%). At the physiological maturity stage, sunflower showed a higher dispersion of disease severity data (27%) than at full maturity (10%). It suggests that the disease severity data could be scored with higher precision at the physiological maturity stage, and, moreover, it would be easier to differentiate genotypes by the SEV-40dai performance because of a wider range of data values.

Another advantage of SEV-40dai over SEV-Mx could be related to the probability of rejecting the null hypothesis, when it is true, referring to the absence of significant differences among cultivars in the analysis of variance. In our experiment, we rejected the null hypothesis of the equality of genotype responses to both disease severities. However, the level of probability was much lower for SEV-40dai (α=0.000%) than for SEV-MX (α=0.048%). Therefore, in order to minimize the risk of incurring a statistical type I error, *i.e.*, rejecting a truly null hypothesis, it seems more reasonable to choose SEV-40dai with a smaller "α" despite SEV-Mx.

Considering DGR and SEV-40dai, which one is more appropriate for describing sunflower genotypic performances at the intermediate and final phases of white rot development? The Spearman correlation value between DGR and SEV-40dai of $r_S=0.59$, significant at $\alpha<0.05$ (Table 2), indicated that the two variables were directly related. Therefore, they should not be used together and one of them should be discarded.

If comparison of SEV-40dai and DGR is desired, the relative rotted area in the inflorescence must be scored at different dates. In the field this could be easily done by feeling the head through the paper bags with fingers, as we did, or by visual inspection, in the case of uncovered inflorescences. With practice, scoring the rotted area of the head does not take too much time or money. The disease severity could be then calculated by a simple mathematical operation (*i.e.*, division) that implies knowing the portion of rotted area in the total head surface.

If SEV-40dai is evaluated, there are some advantages related to the number of scored data as well as to the level of precision of estimation. In our work, the SEV-40dai was estimated once, when the sunflower crop reached the physiological maturity stage known as R9 (Schneiter and Miller, 1981) or M2 (Cetiom, 1992). For estimating DGR, one to three additional observations of disease severity are needed before the crop maturity is reached (M4 stage, CETIOM, 1992). In relation to the precision of data, our results showed that there is no controlled variability for SEV-40dai (CV=2%, Table 1), which is four times lower than that for DGR (CV=10%).

High heritability and relationship of the character with the variables estimating initial stages of white rot development are two advantages related to DGR. The degree of genetic determination, a manner of evaluating the wide-sense heritability (Kearsey and Pooni, 1996), for the DGR (0.54, Castaño and Giussani, 2006) was two times higher than the one calculated for SEV-40dai (0.18, Russi *et al.*, 2004). In the present work the correlation coefficients (Table 2) indicated the null relationship between the DGR and either the DI or RIP. The SEV-40dai was significantly ($\alpha<0.05$) associated ($r=-0.52$) with the RIP suggesting some common genes controlling both characters. The higher proportion of genetic effects controlling the speed of white rot progression after the occurrence of first symptoms as well as the lack of association with DI and RIP suggest that DGR could be preferred over SEV-40dai.

The fact that the environmental effects modify less the magnitude and expression of DGR in relation to SEV-40dai, helped to choose DGR. In this sense, the selection efficiency of genotypes having slow expansion of the disease in the last steps of the white rot development could be increased. The independence of DGR from DI and RIP enhances the probability of detecting individuals having separate resistance genes for each variable by traditional or biotechnological protocols.

In conclusion, the description of genotype reaction to *S. sclerotiorum* on the head will be more complete and efficient if DI, RIP and DGR are simultaneously considered. If these three components are regarded, the sunflower crop resistance would be more complex and the partial resistance would operate better than if only a single variable is involved, because different phases of the disease development are considered. In the field, the selected genotypes could have fewer diseased plants as well as slower disease development than the less resistant ones. Therefore, the level of resistance to white rot could be increased in a short period of time in Argentina or elsewhere.

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DISSECTION DE LA POURRITURE BLANCHE DES CAPITULES DE TOURNESOL DANS SES COMPOSANTS DE LA RESISTANCE PARTIELLE

RÉSUMÉ

La pourriture blanche est une maladie d'importance chez le tournesol. Des nouveaux génotypes doivent être évalués avec précision par leur performance face la maladie avant être libérés au public. La maladie peut être appréciée par des composants de la résistance partielle. Dans cet article nous voulons montrer quels des variables ou composants seraient les plus appropriés pour décrire le comportement du tournesol durant le cycle de développement de la maladie. Douze génotypes ont été évalués dans un seul essai. Après l'inoculation, l'incidence (DI), la période relative d'incubation (RIP), la sévérité de la maladie, observée aux 40 jours (SEV-40ddi) et à maturité (SEV-Mx), ainsi que le progrès de la lésion (DGR), ont été estimés. Des réponses significatives ont été détectées pour toutes les variables. La DI et RIP furent confirmés comme composants de la résistance. Les résultats ont aussi indiqué que le DGR pourrait être considéré comme l'autre variable à user en simultanée avec DI et RIP. Si ces trois composants seraient utilisés dans la sélection des nouveaux hybrides, ces nouveaux cultivars auront une résistance plus complexe parce que la résistance à toutes les phases du développement de la maladie sont mises en jeu.

DISECCIÓN DE LA PODREDUMBRE BLANCA DE CAPÍTULOS DEL GIRASOL EN SUS COMPONENTES DE RESISTENCIA PARCIAL

RESUMEN

La podredumbre blanca es una enfermedad importante en girasol, por lo que durante el desarrollo de cultivares se debe evaluar con precisión las respuestas de los híbridos bajo selección. La utilización de componentes de la resistencia parcial a dicha enfermedad ayudaría a justipreciar mejor los genotipos a los ataques de Sclerotinia sclerotiorum en capítulos. En este artículo se muestran qué componentes serían los más convenientes para utilizar y describir el mérito de los materiales genéticos durante el desarrollo de la enfermedad. Doce genotipos fueron evaluados en un experimento. Luego de la inoculación, se les estimó la incidencia (DI), el período relativo de incubación (RIP) y la severidad de la enfermedad, evaluada a los 40 días (SEV-40ddi) y a madurez (SEV-Mx), así como el progreso de la lesión (DGR). Hubo variabilidad significativa de respuestas. La DI y RIP fueron confirmados como componentes de la resistencia parcial. Los resultados también indicaron que DGR, apreciando las etapas intermedias y finales del desarrollo de la enfermedad, sería – entre las restantes evaluadas en este experimento la variable más deseable a utilizar simultáneamente con DI y RIP. Si éstos tres componentes fueran utilizados al desarrollar nuevos híbridos, los cultivares liberados poseerán una resistencia más compleja habida cuenta que la resistencia a todas las fases de la podredumbre blanca de capítulos estarían controladas genéticamente.