PEDIGREE SELECTION FOR SUNFLOWER CAPITULUM RESISTANCE TO *SCLEROTINIA SCLEROTIORUM*.

Felicity Vear, **Frédéric Serre**, **Pascal Walser**, **Henri Bony**,
Georges Joubert, **Denis Tourvieille de Labrouhe**
I.N.R.A., G.R.E.A.T, Station d'Amélioration des Plantes et de Pathologie végétale, Domaine de Crouelle, 63039 Clermont Ferrand cedex 2, France, E-mail : vear@clermont.inra.fr

Summary:

Pedigree selection programmes to improve capitulum resistance to *Sclerotinia sclerotiorum* in sunflower are carried out with ascospore tests on F2 and F4 generations and mycelium tests on F3 plants. To judge whether selections from crosses of lines with some *Sclerotinia* resistance can give improved reactions, four to six F3 families and the parental inbreds of five crosses were crossed to a tester inbred. The reactions of the hybrids to semi-natural *Sclerotinia* capitulum attack and of F4 families to ascospore tests were compared. The parental lines had been studied earlier and ranged from susceptible to highly resistant. The results show that, in all cases, there was variation in level of resistance and possible gains among the F3 families, compared with their parents, varied from 24 to 61%. The means of the parental hybrids and their F3 hybrids were correlated significantly. Choice between F2 plants according to their reaction to the ascospore tests appears to give a slight gain. Results of ascospore tests on the F4 progeny were not significantly correlated with reactions to semi-natural attack, but provide additional data for choice of progenies to be retained.

Résumé :

Les programmes de création de lignées de tournesol résistantes aux attaques de *Sclerotinia sclerotiorum* sur capitule mettent en œuvre des tests "ascospores" sur les générations F2 et F4 et un test "mycélium" sur les plantes F3. Les niveaux de résistance ont été estimés en infection semi-naturelle à travers des croisements des plantes F3 réalisés avec des testeurs d'une part, et à partir des observations des tests " ascospores " réalisés sur les descendances F4. Les lignées parentales ont été analysées précédemment et classées de sensibles à très résistants. Les résultats montrent dans, tous les cas, une variation du niveau de résistance. Les gains de résistance pour les familles F3, par rapport aux parents, varient de 24 à 61%. Les moyennes des hybrides parentaux et de leurs descendances F3 sont corrélées significativement. Le choix parmi les plantes F2 selon leur comportement au test " ascospores " n'apporte qu'un gain limité. Les résultats des tests " ascospores " sur les descendances F4 ne sont pas corrélés significativement avec les observations en infection semi-naturelle, mais apportent des informations complémentaires pour le choix des descendance à retenir.

Introduction

Pedigree selection programmes for sunflower resistance to capitulum attack by *Sclerotinia sclerotiorum* can be based on resistance tests using infection by ascospore suspensions (Vear and Tourvieille, 1989) on individual plants and families in the course of fixation and on observation of semi-natural attack (Vear and Tourvieille, 1987) of hybrids between tester inbreds and, for example, F3 or other generation, families. Vear and Tourvieille (1989) found that the results of the ascospore test were significantly correlated with those of semi-natural attack, but with a determination of only 33%, indicating that the resistance factors measured are not entirely the same. All the studies on the inheritance of *Sclerotinia* resistance have shown that this resistance is quantitative and under predominantly additive control (Vear and Tourvieille, 1989; Tourvieille and Vear, 1990, Castaño *et al*., 1992.), so breeding programmes have the objective of combining favourable genes from different sunflower genotypes with good S*clerotinia* reactions. A recurrent selection programme based on ascospore and mycelium tests on capitula showed a gain of 10% per cycle for the ascospore latency index and 19% per cycle for the mycelium test, over 8 cycles (Vear *et al*., 1992).

Recent research to determine quantitative trait loci (QTL) for *Sclerotinia* resistance, in crosses from three different lines, showed that there were at least 4 regions concerned (Mestries *et al*., 1998) whose importance varied according to the inbred line used as resistance source. However one QTL appeared particularly important (Gentzbittel *et al*., 1998) and the question was raised as to whether, in breeding programmes, lack of increase in resistance level in the progenies from some crosses might be because the parents contain the same alleles contributing to resistance.

To obtain a first answer to this question, some progenies in a pedigree selection programme were studied in comparison with the inbred lines that had been used to make the crosses from which they had been bred. Hybrids between a tester inbred and the parents and the F3 families were studied under semi-natural attack. In addition, a comparison was made between these results and those from ascospore tests on F2 plants, F4 families and the inbred lines.

Materials and Methods

Sunflower genotypes:

The genotypes studied are listed in Table 1. All the lines are male fertility restorers bred by INRA, with apical branching except for PW3, PWW4 and SDPAC1..134. PSC8 is considered to have very good *Sclerotinia* resistance, both in tests and under semi-natural attack, PST2, PST3 and 83HR4 generally show quite good resistance, PSS2 and PWW4 are intermediate and PW3 is susceptible. SD.PAC1.134 was one of the best F3/F4 families studied in the QTL programme (33% ascospore attack when the mean was 81%; Mestries *et al*., 1998). An example of data from semi-natural attack trials for hybrids with the inbred HA821 as tester was the following:

	1995	1996
PW3	45%	84%
PSC8	19%	55%
PST3	30%	
83HR4	39%	
PWW4		73%
PSS2		72%

The inbreds used as testers, GB and ZF, are CMS lines quite susceptible to *Sclerotinia*.

Table 1. Percentage semi-natural attack and ascospore test results for sunflower-inbred lines and F3/F4 developed by pedigree selection.

Genotype	% semi-natural attack 1999 hybrid GB x	mean	1998 ZF x	ascospore test latency index F2 pl.	F4 mean	% infect. F4 mean
PSS2	34.3	33.1		(118)		(16)
PST2	31.8			(104)		(50)
(PST2 PSS2) F3 (a)	38.5			NS	78a	100
(PST2 PSS2) F3 (b)	38.7	27.9		NS	70a	95
(PST2 PSS2) F3 (c)	18.5			132	81ab	95
(PST2 PSS2) F3 (d)	23.5			NS	89 bc	100
(PST2 PSS2) F3 (e)	20.1			143	90 c	95
lsd	*16.1*					
PSS2	34.3	31.1		(118)		(16)
PWW4		27.8				
(PSS2 PWW4)F3 (a)	30.8			NS	51a	100
(PSS2 PWW4)F3 (b)	15.8	27.4		122	85 b	95
(PSS2 PWW4)F3 (c)	37.9			152	73 b	94
(PSS2 PWW4)F3 (d)	25.1			NS	75 b	100
lsd	*28.7*					
PSS2	34.3	25.1		(118)		(16)
PSC8	15.8		39.0	(120)		(16)
(PSC8 PSS2)F3 (a)	9.8			NS	89a	58
(PSC8 PSS2)F3 (b)	22.9	24.1		NS	107 bc	73
(PSC8 PSS2)F3 (c)	24.5			NS	104abc	69
(PSC8 PSS2)F3 (d)	33.8			NS	97ab	95
(PSC8 PSS2)F3 (e)	32.9			NS	111 c	73
(PSC8 PSS2)F3 (f)	24.0			NS	90a	100
lsd	*28.7*					
PST3	29.4	29.1		(123)		(7)
PW3	28.8		65.9			
(PST3 PW3)F3 (a)	36.4			NS	79 b	82
(PST3 PW3)F3 (b)	59.5*	36.7		NS	68a	100
(PST3 PW3)F3 (c)	35.1			155	80 b	84
(PST3 PW3)F3 (d)	22.1			NS	67a	100
(PST3 PW3)F3 (e)	30.5			164	72 ab	100
lsd	*26.6*					
SDPAC1..134	70.3	56.6	29.3	(080)		(33)
83HR4	42.9					
(SDPAC1..134 83HR4)F3 (a)	35.5			NS	101 b	90
(SDPAC1..134 83HR4)F3 (b)	42.1	47.0		145	85a	63
(SDPAC1..134 83HR4)F3 (c)	62.9			NS	94ab	71
(SDPAC1..134 83HR4)F3 (d)	28.9*			NS	104 b	91
(SDPAC1..134 83HR4)F3 (e)	65.5			NS	86a	88
lsd	*27.2*					

() values in brackets are for inbred lines; * significantly different from parental mean; lsd P > 0.95

F2, F3 and F4 generations were obtained by selfing, following a pedigree selection programme.

Semi-natural attack trials:

These used the methodology described by Vear and Tourvieille (1987). Hybrids were placed in randomised block trials with 50 plants per plot. Irrigation maintained humidity throughout flowering, and, in 1999, there was, in addition, about 120mm rainfall. The numbers of plants showing *Sclerotinia* symptoms on capitula in each plot were counted at physiological maturity.

Ascospore tests :

These followed the method of Vear and Tourvieille (1988). Percentage attack was calculated on the F4 families from 1 replication of 20-25 plants. Latency indices (mean delay in symptom appearance compared with controls) were calculated for single F2 plants and for F4 families. Since, between each family, there were differences in the number of plants showing symptoms, for which the latency index could be calculated, t-tests were used to determine significant differences between indices.

Results

Results are presented in Table 1. The hybrids with tester GB were observed in 1999 (Riom, Puy-de-Dôme department, France), the hybrids with ZF in 1998 (CETIOM, St.Florent, Cher departement, France). The ascospore tests on F2 plants and on the inbred lines were made in 1997. The values for the inbred lines, given in brackets, are means of 2 replications of 25 plants. The tests on the F4 plants were made in 1999 (Clermont-Ferrand, Puy-de-Dôme, France).

The general level of attack of the hybrids made with the tester inbred GB was similar to that of 1995 and lower than 1996. GB x PSC8 showed its expected good level of resistance whereas GB x PW3 was less attacked than expected. Variation coefficients for these trials varied from 22 to 46%, which is not excessive for *Sclerotinia* trials The parental and F3 hybrids developed from SD.PAC1..134 and 83HR4 showed unexpectedly high levels of attack.

All the crosses showed variation between the F3 hybrids, in some cases significant (PSS2-PST2, PST3-PW3, SDPAC1..134-83HR4) in others not (PSS2-PWW4, PSC8-PSS2). In Table 1, in the column " mean ", are given the means of the two parental hybrids and of the F3 hybrids, for each cross. The asterisks indicate F3 hybrids significantly different from the mean of the parental hybrids. When the parental hybrids had similar results (PSS2-PST2, PSS2- PWW4) the F3 hybrids were positioned on both sides of the parental mean. When the parental hybrids were quite different (PSS2-PSC8, SD.PAC1..134-83HR4) the F3 hybrids were intermediate or better than those from the more resistant parent. For PW3-PST3, with one exception, the F3 hybrids were more susceptible than the parental hybrid mean.

For each cross, the means of the F3 hybrids were similar to, or more resistant than, the means of the parental hybrids. The correlation between these two means for each cross was significant (r=0.87). The maximum gain observed ((mid-parent- best F3)/mid-parent) varied from 24% (PST3-PW3) to 61% (PSC8-PSS2).

Comparison of parental hybrids with results available for crosses with the tester line ZF, confirm the reactions of PSC8 and PST3 but SDPAC1..134 gave a much better level of resistance with this tester than with GB.

The ascopore test data for F2 plants shows that all the F2 plants retained showed good reactions, either no symptom at all (NS) or very long latency indices. There was no evident difference between the two types of reaction in following generations. Under the favourable conditions of 1999, the F4 families showed high levels of attack, only those from the crosses PSS2-PSC8 and SDPAC1..134-83HR4 had significantly less than 100% symptoms. This percentage attack was not significantly correlated with percentage semi-natural attack of hybrids, but the same progeny had the lowest level of attack in both cases: (PSS2-PSC8)F3(a).

There were significant differences in latency indices between F4 progenies from each cross, but although this character was indicative of the level of semi-natural attack for SDPAC1..134-83HR4 and PSS2-PSC8, the general correlation was not significant. The correlation between the latency index and percentage infection for the F4 families was r=-0.53 (ns)

Discussion

The semi-natural attack data were obtained from single trials in 1999. There were significant differences between hybrids and the cv were not particularly high, but for fixed inbred lines, it would be normal to carry out further trials to obtain confirmation of results. However, in the course of breeding programmes, with segregating generations, it is necessary to make a choice among material after each year of trials, and confirmation is obtained in later years only on the genotypes retained. The system of comparing the F3 hybrids with parental hybrids, in addition to commercial variety controls, should help in selection by validation of the general level of resistance observed in the F3 hybrids, and by defining gain in resistance.

Since the results of the ascospore test are not closely correlated with those of semi-natural attack trials, they can be used, as an additional result, to confirm choice of progeny to be retained, rather than to replace semi-natural attack observations. The use of percentage infection from the ascospore test will depend on the intensity of attack. When intensity is not great, the plants with apical branching may dry rapidly and escape *Sclerotinia* symptoms, whereas, when disease intensity is high, as in 1999, and many apically branched progeny are 100% infected, those that show less infection may be considered as having good resistance. As an example, when several F3 hybrids have similar percentages of semi-natural attack, the F4 families with the highest latency indices can be retained.

The cross that raises a question is SDPAC1..134 x 83HR4, since the parental lines were considered as highly and quite resistant respectively and the hybrid ZFxSDPAC1..134 showed good resistance in 1998, whereas both the parental and F3 hybrids with the tester GB are among the most susceptible. The latency indices suggest that of the five F4 progenies, (a) and (d) are the best, and this is also true for the F3 hybrids. In this case, it will be necessary to repeat observations of this material to determine whether the susceptibility is a year effect, or an interaction with the genotype GB.

Concerning the genetics of resistance, the results confirm additive control, since the F3 means are correlated with the parental means. The fact that the F3 mean is generally slightly

better than the parental mean suggests that choice of F2 plants has had a small favourable effect. For PST3-PW3, the most likely explanation of the relatively poor F3, compared with the parents, is that, for some reason in 1999, the hybrid with PW3 escaped attack, compared with 1995-96, so the 1999 result was not a good indication of its low resistance level.

Overall, there is no indication, in the crosses studied, that the resistance genes are exactly the same. In all cases, selection of genotypes with improved *Sclerotiona* resistance, compared with the parents, appears possible.

References

CASTAÑO F., HEMERY-TARDIN M.C., TOURVIEILLE de LABROUHE D., VEAR F., 1992. The inheritance and biochemistry of résistance to *Sclerotinia sclerotiorum* leaf infections in sunflower (*Helianthus annuus* L.). Euphytica, **58**, 209-219.

GENTZBITTEL L. & MOUZEYAR S., BADOUI S., MESTRIES E., VEAR F., TOURVIEILLE de LABROUHE D., NICOLAS P.,1998. Cloning of molecular markers for disease resistance in sunflower, *Helianthus annuus* L. Theor.Appl. Genet., **96**, 519-525.

MESTRIES E., GENTZBITTEL L., TOURVIEILLE de LABROUHE D., NICOLAS P., VEAR F., 1998 Analyses of quantitative trait loci associated with resistance to *Sclerotinia sclerotiorum* in sunflowers (*Helianthus annuus* L.) using molecular markers. Molecular Breeding, **4**, 215-226.

TOURVIEILLE de LABROUHE D., VEAR F., 1990. Heredity of resistance to *Sclerotinia sclerotiorum* in sunflowers. III - Study of reaction to artificial infections of roots and cotyledons. Agronomie, 10, 323-330.

VEAR F., TOURVIEILLE de LABROUHE D., 1987. Test de résistance au mildiou chez le tournesol : nouvelles techniques de conservation de l'inoculum. Inf. Tech. CETIOM, 98, 19-20.

VEAR F., TOURVIEILLE de LABROUHE D., 1988. Heredity of resistance to *Sclerotinia sclerotiorum* in sunflower. II. Study of capitulum resistance to natural and artificial ascospore infections. Agronomie, 8, 503-508.

VEAR F., TOURVIEILLE de LABROUHE D., CASTAÑO F., 1992. Recurrent selection for sunflower capitulum résistance to attack by *Sclerotinia sclerotiorum*. Proc. 13th Int. Sunflower Conf. Pise, Italy, 1275-1280.