PARTIAL, NON-RACE-SPECIFIC RESISTANCE TO DOWNY MILDEW IN CULTIVATED SUNFLOWER LINES

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Abstract

Observations of natural attack of cultivated sunflower lines by downy mildew under irrigation have shown a wide range of reactions in the absence of efficient major genes. These reactions are independent of pathogen race and environmental conditions. The use of partial resistance in addition to major genes to obtain more durable control of sunflower downy mildew is discussed.

Résumé

Des observations d'attaques naturelles, sous irrigation, de Mildiou dû à *Plasmopara halstedii,* sur une importante série de génotypes de *Helianthus annuus* cultivés, ont permis de mettre en évidence une très large gamme de comportement, même en absence de gène de résistance spécifique *Pl* efficace. Ce comportement est indépendant du pathotype présent sur la parcelle. Le classement des génotypes est stable quelles que soient les conditions pédoclimatiques. L'utilisation de cette résistance, en complément de la résistance apportée par les gènes majeurs *Pl* dans les programmes de sélection est discutée.

Introduction

Downy mildew (*Plasmopara halstedii* (Farl.) Berl. and de Toni), an important parasite of sunflower (Jouffret et al., 2000) is generally controlled, at present, by the use of varieties carrying major genes for resistance denoted *Pl.* These genes give complete resistance during the whole plant cycle (Vear et al., 2000) and are dominant (Vranceanu and Stoenescu, 1970), so that only one parent need carry an efficient resistance gene for the hybrid variety to be resistant. Thus breeders have made wide use of such resistance to control downy mildew. However, as for most complete monogenic resistances, *Pl* genes do not provide durable resistance because the parasite, *Plasmopara halstedii* shows physiological races (Roeckel-Drevet et al., 2000). Breeders have already used at least 10 genes obtained from both cultivated sunflower and wild *Helianthus* spp. to counter these races (Vranceanu et al., 1981; Miller and Gulya, 1991; Vear et al., 2004). This system will probably not be efficient in the long run, as the pathogen shows continuous evolution with the appearance of new virulences which are selected by the presence of *Pl* genes in the sunflower crop, and new races become important with increasing rapidity (Escande and Peyrera, 2003; Penaud et al., 2003). Breeders must continuously search for new genes. In addition, chemical treatment of seed is no longer effective; *P. halstedii* isolates resistant to metalaxyl are now widely observed (Albourie et al., 1998; Molinero-Ruiz, et al., 2000).

It therefore appears important to make a search for genetic resistance that is not based on *Pl* genes only, but also for partial polygenic resistances that are not race-specific and should therefore be more durable. With this aim, the reactions of 50 inbred lines and a few hybrids were observed in the field with irrigation providing conditions favouring natural infection by the downy mildew race present in the area.

Materials and Methods

Sunflower Genotypes. The inbred lines and hybrids studied were chosen as representative of the known diversity of cultivated sunflower, and because they did not carry major genes giving resistance to the races thought to be present in the trial locations. The three hybrids tested, together with their parental lines, were chosen because they had already been observed in previous years. In addition to this basically susceptible material, the differential lines defined by Gulya et al. (1998) were observed to characterise the downy mildew races present in each location. All the genotypes are listed in Table 1.

Experimental Plan. In each location, the genotypes were sown, and observed, at a density of 100,000 plants/ha. There were three to five replications and plots of 40 to 60 plants. To create conditions favourable for downy mildew infection, an irrigation of at least 50 mm was provided five days after sowing (Délos et al., 2000) and there were further irrigations according to trial and environmental conditions. There were two basic trial plans: 1) INRA, Clermont-Ferrand, the 57 genotypes were repeated under three levels of irrigation; high= 150 mm 8 days after sowing, then 7.0 +/- 1.5 mm per day; medium= 75 mm 8 days after sowing, then 5.0 +/- 1.5 mm per day; and low= 50 mm 8 days after sowing, then 4.0 +/- 1.5 mm per day, 2) at each of the three additional sites which provided a quite good coverage of the sunflower crop in France (Pioneer, Toulouse; RAGT, Rodez and Syngenta, Toulouse), 20 of the 57 genotypes were studied under one irrigation level. Further trial locations were sown but did not show significant downy mildew infections.

Observations were made of the numbers of seedlings which emerged and then the numbers of plants showing systemic downy mildew symptoms: dwarfing, yellowing of leaves and sporulation (Tourvieille et al., 2000). These observations were made at least every two weeks from emergence to the flower bud stage.

Table 1. Characteristics of the 57 cultivated sunflower genotypes observed for their "horizontal" resistance to downy mildew.

Genotype	Type	*Pl* gene	Genotype	Type	*Pl* gene	Genotype	Type	*Pl* gene	Genotype	Type	*Pl* gene
HD37	H	Pl2	B11A3	F	-	TS	F	-	PAZ2	M	Pl2
HD38	H	Pl2	BB	F	-	WH	F	Pl1	PIR2	M	Pl2
HRIG	H	Pl2	CANP3	F	-	*1V1*	M	Pl2	PSC8	M	Pl2
IN65	H	-	CERN51	F	-	*PAC1*	M	Pl2	PSS2	M	Pl2
REMI	H	Pl2	F125	F	-	***PMI3***	M	Pl ?	PST5	M	Pl2
GB	F	-	OA	F	-	*PR56*	M	Pl2	PSU7	M	Pl2
GU	F	-	FN	F	-	***QHP1***	M	Pl ?	PSY4	M	Pl2
H52	F	-	FU	F	-	***RHA 265***	M	Pl1	PSY8	M	Pl2
HA89	F	-	GH	F	-	***RHA274***	M	Pl2	RHA 266	M	Pl1
YDQ	F	Pl6	HIR34	F	Pl 4	LR4	M	-	RHA 348	M	Pl2
RLM1	F	-	JT	F	-	2V13	M	Pl1 Pl2	RHA 428	M	Pl ?
RLM2	F	-	LR1	F	-	83HR4	M	Pl2	YSQ	F	Pl5
SL72	F	-	LR2	F	-	HA61	M	Pl2			
SL82	F	-	SCD	F	-	MRI	M	Pl2			
CAR	F	-	SD	F	-	PAC2	M	Pl2			

Bold type: race-differential lines. *Italics*: genotypes observed in several locations. H = hybrid; F = female; M = male.

Results

The mean percentages of plants showing downy mildew symptoms varied, according to location, from 20 to 58%. The differential lines which contained efficient *Pl* genes were always attacked at less than 10%. For the genotypes without efficient *Pl* genes, the mean level of attack varied from about 15% to 40 to 70%. Different reactions of the differential lines indicated that race 710 was present at Clermont-Ferrand (the line PMI3 had 66% attack, whereas the line QHP1 had only 2%) but in the three other locations, the trials were infected by race 703 (PMI3: 0%, and QHP1: 54%).

In the conditions of abnormal drought in the spring of 2003 at Clermont-Ferrand, the amounts of irrigation largely influenced the mean levels of attack on all the genotypes with no efficient *Pl* gene: 64.9% under the high level of irrigation to 29.8% under the low level of irrigation. However, there was no significant interaction between genotype and irrigation level (Table 2).

The mean levels of attack observed on the genotypes with no efficient *Pl* gene varied according to trial location from 33.4% to 42.3%. The significant correlation coefficients (Table 3) indicate that the 20 common genotypes behaved similarly in the different locations. To determine whether *Pl* genes that do not give effective resistance have any residual effect, the 25 genotypes with no *Pl* gene were compared with the 26 carrying either *Pl1, Pl2* or *Pl4*. The results in Table 4 show no differences between the two groups.

Table 2. Correlation coefficients between level of irrigation during germination and percentage of downy mildew attack on 51 sunflower genotypes not containing efficient *Pl* genes (race 710).(**: p>0.001).

Irrigation level	Percentage attack	Correlation coefficient	
		75 mm	**50 mm**
150 mm	64.91 %	0.768**	0.757**
75 mm	38.23 %		0.780**
50 mm	29,75 %		

Table 3. Correlation coefficients between the percentage of downy mildew attack observed in the four locations for 16 genotypes with no efficient *Pl* gene.

Location	Percentage attack	Correlation coefficients		
		Pioneer	**RAGT**	**Syngenta**
INRA	39.3 % (1)	0.635**	0.842**	0.779**
Pioneer	42.3 %		0.490	0.460
Ragt	37.3 %			0.730**
Syngenta	38.3 %			

(1) Mean of five trials **: p>0.001

Table 4. Comparison of the mean percentage of downy mildew attack of 25 sunflower genotypes with no *Pl* gene and of 26 genotypes which carried a non-effective resistance gene.

Percentage attack	**25 genotypes with no *Pl* gene**	**26 genotypes with a non-effective *Pl* gene**
Mean	37 %	45 %
Max. genotype mean	61 %	71 %
Min. genotype mean	17 %	13 %

To determine whether the differences observed between sunflower genotypes were independent of downy mildew race, the relative reactions of the 16 lines or hybrids not carrying any efficient *Pl* gene in the four trials at Clermont-Ferrand where race 710 was present, were compared with the relative behaviours of these genotypes in the three trials in southwest France where race 703 was present. The correlation coefficient was r= 0.897 (p>0.001), indicating that the partial resistance measured was equally efficient against races 710 and 703.

Table 5 presents the mean results, over all the trials, for each of the 51 genotypes with no efficient *Pl* gene and the relative reaction of these genotypes to *Sclerotinia sclerotiorum* (Lib.) de Bary capitulum attack. There does not appear to be any relation between partial resistance to downy mildew and partial resistance to *S. sclerotiorum.*

Discussion

These results indicate that the observations made in the field did concern a partial, quantitative resistance to downy mildew which is independent of pathogen race. The levels of attack depended on environmental conditions, in particular humidity, but these did not appear to cause any significant changes in ordering of genotype reactions.

These results also show that partial, polygenic non-race-specific resistance is present in modern cultivated sunflower inbred lines, such that it is already, although unintentionally, used by breeders. This resistance probably explains differences already observed in the field (Ljubich, 2000; Al-Chaarani, 2002) but the present results indicate that it is most important to

determine the downy mildew races present in field trials and the *Pl* genes present in the sunflower genotypes studied. This partial quantitative resistance to downy mildew appears to be different from that concerning *S. sclerotiorum* capitulum attack. This is not surprising since the two pathosystems are quite different. *S. sclerotiorum* is an ascomycete and a facultative parasite with a wide host range, whereas *P. halstedii* is an oomycete and an obligate parasite specific to sunflower.

Since the inbred lines studied are reasonably representative of the variability presently used in sunflower breeding programmes, it may be suggested that such programmes carried out over the last 40 years to introduce *Pl* genes have not eliminated partial resistance genes and thus it should be possible to breed genotypes that are agronomically valid and that carry non-race specific resistance.

Table 5. Mean percentage of plants showing systemic downy mildew symptoms under conditions of natural infection (%DM) and level of resistance to *Sclerotinia sclerotiorum* (Ss) for 51 cultivated sunflower genotypes (17 to 28 replications of 40 to 60 plants).

Genotype	% DM	Ss	Genotype	%DM	Ss	Genotype	%DM	Ss
PR56	12.1 ± 3.0	S	SD	30.5 ± 7.5	R	RHA 274	50.5 ± 6.0	-
RLM2	13.0 ± 3.2	-	F125	31.6 ± 11.0	S	HIR34	51.4 ± 12.6	-
REMI	13.1 ± 2.7	R	PAZ2	31.7 ± 2.9	R	SL72	54.3 ± 6.0	-
BB	17.1 ± 4.7	-	GU	32.4 ± 6.2	S	PST5	55.4 ± 11.3	R
HRIG	17.6 ± 3.8	R	1V1	33.7 ± 8.6	-	HD38	56.3 ± 6.5	-
LR4	21.9 ± 4.7	-	WH	36.3 ± 6.1	-	IN65	56.4 ± 5.0	-
PSS2	23.1 ± 6.4	S	HD37	37.7 ± 5.2	-	CANP3	56.9 ± 9.7	S
FU	23.3 ± 5.0	-	LR2	38.4 ± 9.1	-	RHA 265	58.4 ± 6.0	S
PSY4	23.9 ± 6.5	-	LR1	44.3 ± 9.5	-	2V13	58.6 ± 1.9	-
OA	23.9 ± 8.1	-	GB	44.8 ± 8.6	S	RHA 266	59.1 ± 8.5	S
MRI	24.1 ± 6.7	R	HA61	45.3 ± 9.0	R	GH	60.3 ± 11.0	S
RLM1	25.4 ± 6.2	-	H52	46.1 ± 8.5	-	JT	60.5 ± 7.9	-
SCD	26.5 ± 7.1	R	PAC1	47.4 ± 5.1	R	PSY8	62.1 ± 9.2	-
B11A3	27.6 ± 5.5	-	TS	47.7 ± 7.5	-	PSC8	64.6 ± 8.2	R
FN	28.6 ± 8.3	R	83HR4	49.1 ± 8.1	-	PIR2	65.6 ± 10.1	-
SL82	28.7 ± 5.3	-	RHA 348	50.1 ± 6.3	-	PAC2	66.8 ± 9.7	R
HA89	29.1 ± 7.9	-	CERN51	50.2 ± 8.4	-	PSU7	71.3 ± 5.7	R

S= susceptible to *S. sclerotiorum* capitulum attack; R= good level of resistance to *S. sclerotiorum;* -= intermediate reaction to *S. sclerotiorum.*

The apparent absence of any residual effect of ineffective *Pl* genes (overcome by new pathogen races) suggests that the mechanisms involved are different and that partial resistance does not depend on recognition of the parasite by the plant.

Conclusions

Breeding programmes which take partial downy mildew resistance into consideration should be of greatest importance in providing durable resistance. Combination in one genotype of one or a few major genes giving complete resistance and of several genes (or QTL) for non-race-specific resistance should limit rapid multiplication of new races with increased virulence. In the event that new races did develop, partial resistance would limit economic losses caused by the disease. These preliminary results require confirmation and, to permit their use in practise, considerable further research is necessary. These include: 1) development of a method of measuring partial resistance in the growth chamber, 2) determine

inheritance of this (or these) characters, 3) find phenotypic or, more probably, molecular markers that allow identification of the presence of partial resistance genes in the presence of major complete resistance genes, and 4) long term exploitation of non-race-specific resistance from cultivated and wild genetic resources should significantly improve the durability of downy mildew resistance, re-enforcing barriers against widespread development of new pathogen races.

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