RECENT RESEARCH ON DOWNY MILDEW RESISTANCE USEFUL FOR BREEDING INDUSTRIAL - USE SUNFLOWERS

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Summary

For sunflowers to be a profitable industrial-use crop, input costs must be as low as possible. Genetic resistance is therefore essential to control diseases without any spraying or seed treatment procedures. In France, downy mildew is one of the most potentially important diseases. So far, complete, major gene resistance (*Pl* genes) has been used successfully, but with the appearance of seven new races since 2000, research on more durable resistance has been undertaken. Since 2003, methodologies for large scale trials have been developed to study field reaction to downy mildew attacks on genotypes which do not have *Pl* genes effective against the predominant races present. It has been shown that significant levels of partial resistance exist in cultivated sunflower lines (15% infection when susceptibles show 80-90% infection). This resistance appears independent of race (at least 710 and 703). Heredity is under additive control and the behaviour of hybrids can be quite well predicted from inbred lines. Present and future research programmes are discussed and suggestions are made for the use of partial resistance in breeding programmes.

Introduction

For sunflower to be a profitable source of raw materials for industrial transformation or bio-fuels, this crop must compete with other sources of equivalent composition. Production costs must therefore be reduced as far as possible. It may be that 3-way hybrids will become of increased interest as varietal structures, and crop management may be limited to sowing and harvest. For disease resistance, the variety must defend itself, with no spraying or seed dressing. Thus genetic resistance is essential. For the breeder to be able to concentrate on increasing yield under low input conditions, it is important that he does not have to spend too much money or effort on breeding for disease resistance. Any work he does will have to be durable.

In France the most important or potentially most important diseases are downy mildew (*Plasmopara halstedii*), white rot (*Sclerotinia sclerotiorum)*, Phomopsis(*Diaporthe helianthi*) and Phoma (*Phoma macdonaldii*). Phoma is a quite new problem and studies still concern yield loss and epidemiology, Phomopsis attack has lessened in recent years, probably because breeders have developed varieties with good resistance which appears to be of the partial type, without the appearance of pathogen races. Research on, and breeding for, *Sclerotinia* resistance is certainly still necessary but since this resistance is partial and no interactions with isolates have been found (Vear et al, 2004), it can be considered that the increases in resistance that have been obtained will not be lost in the future. In contrast, in recent years, there has been a continuous chase after new resistances to downy mildew. This resistance, controlled by major genes, denoted *Pl*, is complete but race specific. Since 2000, 7 new *P.halstedii* races have been reported (Moinard et al, 2006), meaning that the reaction of many

varieties that were registered as resistant (or re-registered after introduction of new *Pl* genes) varies according to where they are grown. Breeders have to spend a lot of time back crossing their best inbred lines to introduce new *Pl* genes (Vear 2004).

New *Pl* genes do exist. So far, *Pl* genes have been found to be grouped in clusters, each "gene" (taken in the Mendelian inheritance sense) giving resistance to one or a few races. Three clusters have already been published (Mouzeyar et al, 1995, Bert et al, 2001, Dussle et al, 2004) and studies in progress suggest that at least two more exist. In addition, studies on introgressions and wild *Helianthus annuus* by both USDA and INRA have shown that *Pl* genes are not rare in wild annual *Helianthus* species (T.Gulya, H.Serieys, personal communications). It might be possible to continue to breed sunflower varieties resistant to the main races of downy mildew with genes from these sources, but, if no there is no reflection on their use , it is likely to lead to a waste of time and money *(*Tourvieille, 2004)

Research for durable resistance to sunflower is recent, the best methods are not yet proven. Taking into consideration all the work on model and crop species and the similarities in resistance gene structures between species, it might be possible to identify major resistance gene structures that are more durable than others. Already, it is known that the cluster containing *Pl6*, giving very complete resistance but which has already been overcome by races 304, 314... is of the TIR- NBS-LRR type (Bouzidi et al,2001), whereas the cluster containing *Pl8*, which has not yet been overcome but gives "cotyledon limited susceptibility" is an NBS-LRR-CC (Radwan et al, 2002). However, application of this idea does not appear to be an immediate prospective.

The alternative is resistance of sunflower which does not depend on host/ /pathogen interaction and recognition, so that selection pressure on the pathogen population is much reduced. This type of resistance, usually partial, is what we know for *Sclerotinia*. If resistance levels are sufficient to avoid yield loss it could be used alone. This could be possible for downy mildew attack, which occurs on young plants when there can be compensation by healthy plants, in comparison with *Sclerotinia* capitulum rot which appears just before harvest with no possibility of compensation. However, partial resistance could also be combined with *Pl* genes such that, if spores or mycelium of a new pathotype formed, their multiplication would be so limited that a new race would not develop.

Partial resistance to downy mildew has been studied in France since 2003, in a collaboration programme between INRA, CETIOM and breeders (Oléosem). First results were presented at Fargo (Tourvieille et al 2004).This paper presents the research carried out in the last 3 years, particularly concerning large scale trials, and discusses programmes in progress and possible use of this type of resistance in breeding.

Materials and Methods

Sunflower genotypes

1. Genetic resources : To determine the variability and levels of resistance available in cultivated sunflower, in 2004 and 2005, 820 inbred lines covering all the origins in the collections at INRA, Clermont-Ferrand and some at Montpellier, 73 open pollinated varieties or populations and 59 lines representing introgressions from wild *Helianthus* species, were studied in trials at Clermont-Ferrand. The best lines were observed in trials a second year (2005 and 2006), either at Clermont Ferrand or in multi-location trials in collaboration with French sunflower breeders.

2. Hybrids : From first results in 2003 and 2004 , factorial crosses between 6 females : FRIGA, FU, GU, GX(2006), HA89(2005), H52(2005), IR(2006), SL72 and 5 or 6 restorers (83HR4, 90R18, PAZ2(2006), PR56, PSU7, PUR2(2006), RHA266(2005)) were used to produce hybrids representing the whole range of resistance / susceptibility observed for inbred lines. These hybrids were observed in trials in 2005 and 2006.
3. Checks : inbred lines were chosen from first results in 2003 and confirmed in following years : good resistance B-line FU, restorer PR56, poor resistance : B-line GB, restorer : PSU7. These checks were placed in all trials to permit comparison between years and locations.

Infection Methods and Observations

1. Sowing and Irrigation. Untreated seed were sown with a normal trials/breeding nursery drill at double density (100-120 000/ha) at the normal sowing date for sunflower (late April at Clermont-Ferrand), with 2-row plots of 50-60 plants.
Eight to 10 days after sowing, when radicle length was 2-3cm, complete cover sprinkler irrigation provided at least 60mm. For the following 2-3 weeks further irrigations were made if there was no rainfall (10-20 mm/week).
Trials were made in fields naturally infected with downy mildew; when necessary, to homogenise infection, fresh zoospores from infected plants were added to irrigation water. Downy mildew race was checked by sowing differential lines next to the trials.

2. Observations
Three to 4 weeks after sowing (cotyledon stage), the number of plants emerged in each plot was counted (including those showing symptoms of damping off).
Two to 3 weeks later (2-3 pairs of leaves) , the number of healthy plants per plot were counted (rather than the number of diseased plants since some of these had already withered).
Percentage infection was then calculated from (100-% healthy plants).

Immediately after verification of counts, the trials were rotavated or ploughed and maize, barley or a forage grass were cropped the same year. At the end of the programme, the fields can be disinfected if required.

Results

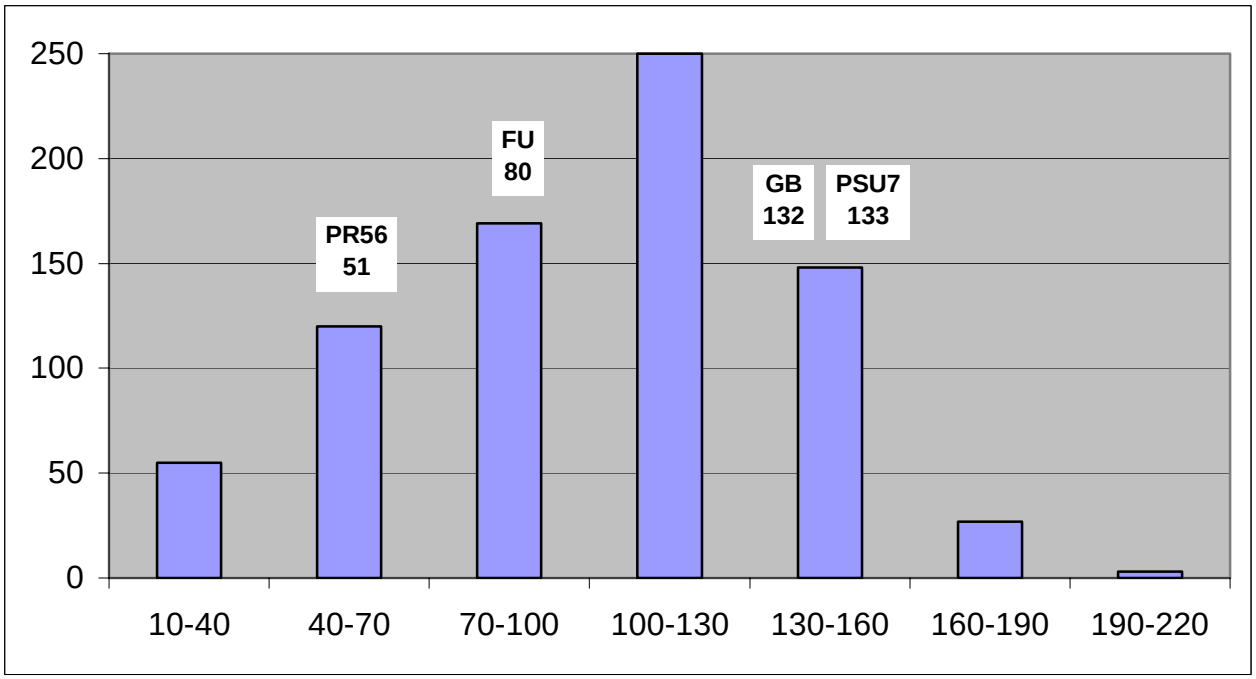
1. Checks
Infection levels varied between years, the means infection levels for the four check inbreds at Clermont-Ferrand are presented in Table 1. The relative reactions of the four lines were quite stable and it is considered that lines with significantly less infection than the mean of these checks present useful levels of partial resistance.

2. Genetic resources
Figure 1 presents the complete range of inbred lines, compared with the checks. About one third of the genotypes show some resistance and 5% (43 inbred lines, 1 population and 1 introgression source) have been confirmed as significantly more resistant than the check mean over two years and, for the 2004-2005 group , in multi-location trials. Table 2 presents an overall analysis of this trial in four locations, one trial with race 703 and three with race 710. Results in the different locations were all highly significantly correlated ($r = 0.45$ to 0.59), in spite of the differences in level of attack. Reaction does not appear to vary with race.

Table 1 : Percentage downy mildew attack in 2003, 2004, 2005 and 2006 on the 4 inbred sunflower lines used as checks for trials since 2004.

lines	2003	2004	2005	2006
PR56	18.16% ± 6.24	31.72 ± 4.37	23.38 ± 3.95	18.97 ± 2.54
FU	28.98% ± 5.09	61.48 ± 4.84	62.23 ± 5.55	31.54 ± 4.37
GB	58.75% ± 10.85	88.21 ± 1.87	81.09 ± 2.53	73.89 ± 6.39
PSU7	73.50% ± 9.48	90.84 ± 2.47	86.23 ± 3.30	74.77 ± 5.66

Figure 1 Distribution of downy mildew attack on nearly 1000 cultivated sunflower genotypes compared with the mean of the 4 check inbred lines indicated



Percentage of attack on mean of check lines

Origins of resistance appear to be quite diverse, among them :

very old lines maintained in our collection since 1970 (e.g. line from Rumanian genic hybrid HS53),

old inbreds dating from 1980-90 (e.g. : INRA : PAH3, PB3, PR56, GX (from BU 1507), USDA : HA 60, HA 113, Canada : CM304, Russia : MO60, MO502, VIR1634),

recent breeding lines (e.g. : INRA : BB, BT, IR (from Impira), FU, USDA : RHA 387, RHA 418, HAR8).

3. Inheritance

In 2005, study of 30 hybrids (6 females x 5 restorers) in 4 locations showed highly significant parental effects (F_f : 5.29**, F_m : 12.18**) and no significant interaction (F_i : 1.96). Infections levels varied from 30% (FU x 90R18) and 32% (FU x PR56) to 83% (HA89 x PSU7 and SL72 x PSU7) for a mean attack of 65%.

For 2006, the results for a 6F x 6R factorial at Clermont-Ferrand are presented in Table 3. Infection level for the two best hybrids in 2005 was slightly lower (FU x 90R18 :

Table 2 . Percentage attack by Downy Mildew in 4 locations in 2005. Check = (GB + FU+PSU7 + PR56)/4. (check mean : 52.5%). T1-T4 : Trial 1 to Trial 4, with downy mildew race identified.

Inbred line	Mean % Attack	% check mean	sig.dif check	T1 710	T2 710	T3 703	T4 710
GB	62.29	118.70	NS	96.66	27.81	75.64	49.04
FU	35.40	67.46	NS	73.97	18.52	36.12	12.98
PSU7	77.60	147.89	SUP	100.00	43.53	74.28	92.61
PR56	34.61	65.96	NS	42.66	39.88	32.01	23.89
83HR4	58.73	111.91	NS	93.22	28.13	75.26	38.29
90R18	28.95	55.16	INF	75.62	5.49	14.25	20.43
AR 1465	37.41	71.28	NS	53.00	25.49	27.34	43.79
BB	26.19	49.90	INF	63.33	9.10	15.23	17.09
BT	30.02	57.20	INF	63.06	22.32	17.67	17.02
BU 928	51.40	97.96	NS	93.90	60.67	36.27	14.77
HA89	45.02	85.79	NS	89.88	32.00	25.38	32.81
CM617xCM620	24.11	45.95	INF	51.18	8.75	26.47	10.05
A1786	19.94	37.99	INF	50.53	3.57	14.56	11.09
DIV.2.	22.99	43.81	INF	57.52	0.00	23.35	11.09
DSCL114	13.84	26.37	INF	39.30	9.20	1.19	5.66
E 474	31.60	60.22	INF	62.99	10.36	26.96	26.10
FN	39.95	76.12	NS	68.33	7.41	68.93	15.11
FRIGA	39.52	75.31	NS	79.90	19.05	25.96	33.16
GU	57.38	109.35	NS	84.89	64.25	66.42	13.97
GX	30.37	57.87	INF	69.87	0.00	38.62	12.97
H52	54.89	104.61	NS	84.21	44.44	30.50	60.42
HAR2	34.57	65.88	NS	73.33	11.64	52.00	1.32
HAR8	19.40	36.97	INF	51.67	6.52	7.18	12.24
IR	31.83	60.66	INF	50.14	10.00	42.58	24.61
K 2528	37.77	71.98	NS	75.11	14.93	32.87	28.18
OA	42.69	81.35	NS	78.60	40.74	12.78	38.64
PAZ2	22.70	43.26	INF	58.61	8.33	15.92	7.94
PSS2	36.62	69.79	NS	68.30	31.32	21.07	25.79
PSY4	53.86	102.64	NS	78.51	25.89	61.26	49.79
PUR2	51.30	97.79	NS	57.22	80.00	32.53	35.45
RHA266	58.94	112.31	NS	81.07	39.15	36.12	79.40
SD	36.87	70.26	NS	85.32	9.62	33.82	18.72
SI	31.95	60.89	INF	63.82	5.65	41.43	16.91
SL72	84.89	161.78	SUP	93.04	86.17	79.81	83.55
U 2169	24.64	46.96	INF	50.76	6.63	27.82	13.35
UCL 81	29.52	56.25	INF	73.89	1.79	22.83	19.55
WG(HA60)	28.95	55.16	INF	63.48	6.67	19.44	26.19
WJR 1624	36.75	70.03	NS	64.73	10.00	46.19	26.08
WJR 1634	21.55	41.06	INF	49.98	2.08	30.02	4.10
WX	38.20	72.79	NS	60.42	31.37	18.14	42.86
General mean : 38.63; lsd = 19.78 ; Location means				69.30	22.71	34.58	27.93

Table 3 : Percentage downy mildew attack (race 710) on a factorial cross at INRA Clermont-Ferrand 2006. Means of 4 replications (check mean : 50.2%)

	IR	**GX**	**FU**	**FRIGA**	**GU**	**SL72**	**Mean M**
90R18	7.6	33.8	21.2	41.8	42.2	66.0	**35.4%**
PR56	16.1	20.2	22.0	37.4	55.7	62.5	**35.7%**
PAZ2	25.2	37.6	40.2	29.2	72.0	46.4	**41.8%**
PUR2	20.5	38.7	51.3	39.9	76.7	60.7	**48.0%**
83HR4	28.8	46.8	39.5	57.5	81.0	71.1	**54.1%**
PSU7	31.8	61.1	65.5	83.8	77.1	91.4	**68.5%**
Mean F	**21.7%**	**39.7%**	**40.0%**	**48.3%**	**67.5%**	**66.4%**	**47.3%**

Blocks F = 2.11ns Females F = 43.35* Interaction F = 1.96ns
Hybrids F = 10.80** Males F = 22.45**

21%, FU x PR56 : 22%) while it was higher for the most susceptible genotypes (SL72 x PSU7 : 91%), for a mean of 47%. This greater difference was probably because the results are for a single trial. Compared with 2005, the main difference came from the introduction of IR, a female line which gave hybrids showing infection levels of only 8 to 32%. Parental effects were very highly significant, and there was no significant female-restorer interaction. From the two years of results, it can be concluded that heredity is under additive control.

Correlations between reactions of inbred lines (Table 4) and the means of their hybrids were significant both for females (r=0.916**) and restorers (r = 0.817,*). It is thus possible to predict the reaction of hybrids from those of parental lines. One slight exception was that the two most resistant lines, IR and 90R18, gave hybrids better than expected from the parental lines.

Table 4 : Percentage downy mildew infection of the inbred lines, parents of the factorial cross, at INRA Clermont-Ferrand 2006. Means of 4 replications (check mean : 51.3%).

Females		*Males*	
IR	27.9%	90R18	34.1%
GX	30.9%	PR56	19.0%
FU	28.3%	PAZ2	28.8%
FRIGA	55.1%	PUR2	32.8%
GU	96.2%	83HR4	89.3%
SL72	76.8%	PSU7	77.3%

Discussion

These results obtained since 2004 are encouraging since they indicate that useful levels of partial resistance exist in a wide range of agronomically valid cultivated sunflower genotypes and that inheritance is relatively simple. Either using the sources we have identified or from search among their own lines, breeders should be able to include partial resistance in future varieties.

The ease of use will depend on the results of two research programmes in progress. Firstly QTL identification, on 3 populations, one F3 and two RIL. These studies should provide not only markers (mostly SSR) but also answer the questions of

the number of QTL involved,

whether they differ according to genetic origin,

whether they are related to *Pl* genes (Tourvieille et al, 2004 found no evidence for this)

It should also open the field for research on candidate genes which would help to decide whether the resistance are truly of the non-race-specific type, and not *Pl*-type genes giving wide-range but partial control of downy mildew.

The objective of the other programme in progress is most important to simplify studies of partial resistance : its measurement by tests on young plants in the growth chamber. This is essential to be able to carry out tests with a wide range of races, some of which must be kept in confinement chambers, to obtain evidence for non-race-specificity. It would also facilitate screening in areas where downy mildew does not occur naturally and at any period during the year, not only when environmental conditions are favourable. It would also be an advantage if it was possible to distinguish partial resistance from segregation for *Pl* genes, at present this is quite difficult .

How can this partial resistance to downy mildew be used in breeding ? It appears possible that it may be sufficient alone to avoid yield loss. The hybrid IR x 90R18 only showed 7 % attack when others has 80-90% of plants with downy mildew symptoms and we have made the combination IR x OPB7 (16% attack when 90R18 had 34%), which will be observed in trials in 2007. Large scale testing will be necessary to conclude on this point. Probably the first method to include partial resistance in new varieties will be to combine *Pl* genes in one parent with good partial resistance in the other. This should at least protect against very rapid development of a new race. To develop new inbred lines with good partial resistance, with or without *Pl* genes, will require molecular markers, basically to identify plants carrying the required QTL, but they would also be useful for *Pl* genes when the reaction of these to seedling tests is difficult to distinguish from good partial resistance.

A good deal of research remains to be done to obtain sunflower varieties with durable resistance to downy mildew, but the first, most important step is for breeders, seed merchants and farmers to understand the importance of this character. The evidence suggests that pathologists, geneticists and breeders will be able to develop the techniques necessary for its inclusion in high yielding sunflowers.

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