Analysis and Location of Sunflower Downy Mildew Resistance Genes

Felicity VEAR, Joël MILON, Jacqueline PHILIPPON, Saïd MOUZEYAR, Paul NICOLAS, Denis TOURVIEILLE de LABROUHE

INRA, GREAT

- Station d'Amélioration des Plantes et de Pathologie Végétale
Domaine de Crouëlle - 63039 Clermont-Ferrand Cedex 2 (France)

- Unité Associée Université INRA - OVGV,
Campus des Cézeaux, 63177 Aubière Cedex (France)

SUMMARY

Downy mildew resistance genes in sunflower were studied by traditionnal genetical analyses of testcross progenies and by analyses of linkage between these genes and RFLP markers. In contrast with some earlier results *Pl4*, giving resistance to races 1 and 2, which originated from a cross between *Helianthus tuberosus* and cultivated sunflower, does not appear to be linked or allelic with *Pl2*, but it has not yet been located on the sunflower linkage map. Similarly, *Pl5*, which also came from *H.tuberosus* but which gives resistance to all French races, does not appear located in the cluster including *Pl1*, *Pl2* and *Pl6*, but remains to be located. This « gene » could be be a cluster like *Pl6*, with several genes giving resistance to different races.

INTRODUCTION

In the past, using traditional genetic analyses of F2 or testcross progenies, most sources of downy mildew resistance were reported to contain single independent genes giving resistance to one or more races (Vranceanu and Stoenescu 1970, Zimmer and Kinman 1972, Vranceanu et al. 1981, Miller and Gulya 1987, Sackston et al. 1990). More recently, segregation was not observed in testcrosses between resistant lines (Mouzeyar, 1993), suggesting that some genes were linked or allelic. These contrasting results made it necessary to search for a different method of studying downy mildew resistance genes and when a molecular map based on RFLP markers became available (Gentzbittel et al., 1995) this appeared a good tool for localising resistance genes, separately one from the others. Analyses involved crosses of resistant lines with completely susceptible lines and downy mildew tests of about 150 F3 families, a solid basis to determine genotypes. The first three genes studied, *Pl1*, *Pl6* and *Pl2* were all found to be linked to the same marker, SUN 124-E2 on linkage group 1 of the Cartisol map (Mouzeyar et al., 1995, Roeckel-Drevet et al., 1996, Vear et al., 1997). Using these large numbers of F3 progenies, another important finding was that *Pl6* is not a 'strong' gene, giving resistance to all races, but a cluster of genes, at least some of which can be separated, resistance to races 1 and D (2?) being about 0.6cM from resistance to French races A, B and C (3?) (Vear et al., 1997).

This is a similar situation to that reported for resistance to downy mildew in lettuce (Farrara et al., 1987, Maisonneuve et al., 1994). However, in the latter case several clusters of genes are known, so it appeared useful to continue studies to determine whether the other *Pl* genes in sunflower were located with *Pl6* or whether they were on different linkage groups.

Although the traditional genetical studies have shown their limits, since the molecular studies take time and means, the first do appear useful in preliminary determination of which resistance sources should be studied in detail. Based on their declared origins from *Helianthus tuberosus*, it was decided to study the genes known as *Pl4* and *Pl5*, which could be different from *Pl1*, *Pl2* and *Pl6*, which all probably originated from wild *H.annuus*. *Pl4* was obtained by Leclercq et al. (1970) from a cross between Armavir 9343 and the Jerusalem artichoke variety D19-6 and gives resistance to races 1 and 2 (D). Vear (1974) found this gene to be independent of *Pl1* and *Pl2*, whereas Zimmer and Kinman (1972) and Mouzeyar (1993), observed no segregation in testscrosses or F2 progenies of *Pl2* with *Pl4*, and considered the two to be the same gene. Miller and Gulya (1987) reported

that *Pl5* was present in the Russian population varieties Progress and Novinka and the Rumanian line Rf 5566-74 . They concluded that this gene gave resistance only to race 3 of downy mildew, whereas Vranceanu et al. (1981) indicated that it gave resistance to races 1, 2 and 3. Although Sackston et al. (1990) made no distinction between the synthetics DM2 and DM3, developed from Novinka and Progress respectively, Mouzeyar et al. (1994) found that there were differences between lines derived from these populations and synthetics. In particular, the line PMI3, derived from DM2, is resistant to races 1, D, C and B in France , but susceptible to race A. This is in contrast to the lines XPQ, from Novinka, XRQ from Progress and QPR2, from DM3, which are all resistant to all the French races, A included.

This paper reports traditional genetical studies and progress in molecular studies to elucidate the position and structure of these genes.

MATERIALS and METHODS

Sunflower genotypes : *Pl4* : The line containing this gene is denoted HIR34. Two forms, with different seed colours are available, one selfed since 1972, with striped seed, and the other backcrossed to give a CMS form, with black seed. The lines were crossed and a testcross made of the F1 with a completely susceptible line DF. In provision for the molecular studies, the black seeded type was crossed with GH, the same completely susceptible line as used by Mouzeyar et al (1995) and Vear et al (1997) for location of *Pl1* and *Pl2*. The F2 and F3 generations were obtained by selfing. The F3 and testcrosses were tested with downy mildew race 1.

Pl5 : The line XRQ, obtained from a cross of HA89 with Progress was crossed with the other lines possibly carrying *Pl5* : XPQ (Novinka) and LC18A (Rumanian). XRQ and PMI3 (from DM2, susceptible to race A) were crossed with HA335 (*Pl6*). The F1 progenies were testcrossed to DF as above. XRQ was also crossed with a line carrying *Pl2*, PSC8 for the location studies and the F2 and F3 families obtained by selfing. Segregating progenies were tested with either or both race 1 and race A of downy mildew.

Downy mildew races : Race 1 is maintained on Peredovik, race A on an experimental hybrid containing *Pl2*, EL64. Race A appears to differ from race 4 by the fact that lines and synthetics such as DM3, developed from Progress, are resistant to the first and susceptible to the second.

Table 1. Segregations for downy mildew resistance race 1 among progenies involving HIR34, the sunflower line carrying *Pl4*.

Progeny	resistant		susceptible	Hypothesis	X^2
(HIR34CMS x HIR34self) x DF (black) (striped) (sus)	182		0	1 gene	--
(HIR34CMS x RHA266) x DF (*Pl1*)	212		81	2 genes	1.093ns
(GH x HIR34black)F3	22 homozyg.	40 heterozyg.	32 homogyg.	1 gene	4.21ns

Downy mildew tests : Tests with races 1 and A were carried out in separate growth chambers using the technique of Mouzeyar et al. (1993). It should be noted that for both resistance origins, plants with cotyledon limited infection (CLI) were observed and considered as resistant.

Molecular Biology : DNA was extracted from F2 plants by the technique of Gentzbittel et al.,(1995). Following the tests of F3 progeny, bulks of homozygous resistant and homozygous susceptible plants were made for Bulked Segregant Analysis (Michelmore et al., 1991). RFLP markers known to be linked to the *Pl1,2,6* region (SUN 124E1-2, SUN017H3-3)or to give general coverage of the known linkage groups (Gentzbittel et al., 1995) were used to study polymorphism of parents and bulks and then for segregation analysis.

RESULTS

a) *Pl4*

The two types of HIR34 were observed to be morphologically the same in the field, and a testcross showed no segregation (Table 1), confirming that they have the same resistance gene and are almost isogenic. In contrast with the results of Mouzeyar (1993), a testcross between HIR34 and RHA266 (*Pl1*) showed segregation agreeing with a Mendelian proportion of 3:1, indicating two independent genes (Table 1).

For the GH x HIR34 cross, race 1 tests on F3 families showed 22 homozygous resistant : 40 heterozygous : 32 homozygous susceptible, which agrees with a Mendelian segregation of 1:2:1, indicating the presence of a single dominant gene. The molecular studies of the parental lines and the bulks of F2 plants showed that the markers which flank the *Pl1,2,6* region were polymorphic between GH and HIR34 but not between the resistant and susceptible bulks (Fig.1). Five other probe-enzyme combinations were found to be polymorphic between the bulks but, unfortunately, these were not mapped by Gentzbittel et al. (1995), so their location is still in progress. Segregation studies of two of them show them to be linked at between 20 and 30cM with *Pl4* but not with each other.

b) *Pl5*

Results of testcrosses with the different sources of *Pl5* are given in Table 2. The crosses of XRQ (Progress), XPQ (Novinka) and LC18A (Rumanian) tested with race A show no segregation, suggesting that these

Figure 1. Status of RFLP markers on sunflower linkage group 1 of the two crosses GH x HIR34 and XRQ x PSC8, indicating absence of linkage to markers in the area where *Pl1*, *Pl2* and *Pl6* were located. NL : markers showing polymorphism between parents but not between resistant and susceptible bulks. -- : markers not polymorphic between parents.

	GH x HIR34 (*PL*4)	XRQ x PSC8 (*PL*5)
Markers		
S017H3-3	NL	NL
S124E1-2	NL	NL
S003H3	--	NL
S075H3-1	--	--
S039H3		--
S145H3-2	NL	NL
S097H3	NL	NL
S037H3	--	--
S031H3	--	NL

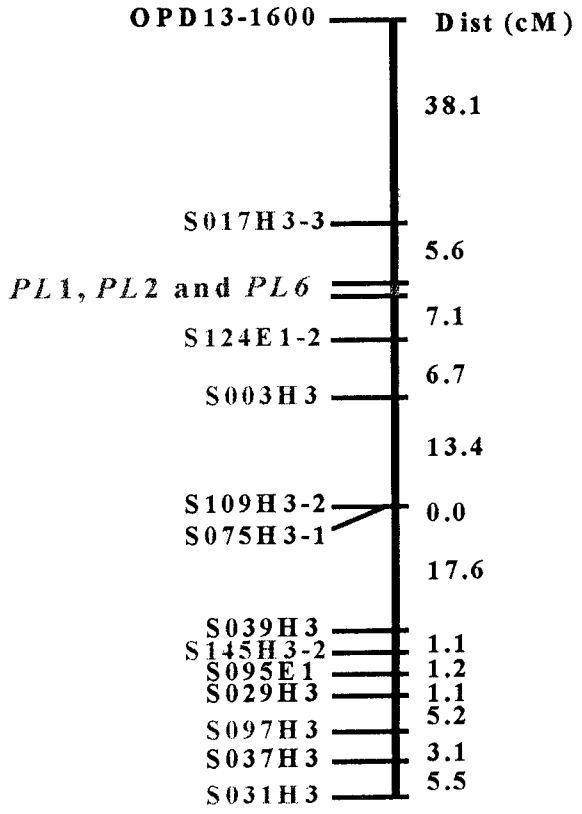

Table 2. Segregations for downy mildew resistance races A and 1 among progenies involving sunflower lines carrying *Pl5*.

Progeny	resistant		susceptible	Hypothesis	X^2
Race A					
(XPQ x XRQ) x HA89 (Novinka)(Progress)(sus)	281		0	1 gene	--
(LC18A x XRQ) x DF (Rum)	284		0 ?	1 gene	--
(XRQ x HA335) x DF (*Pl6*)	202		72	2 genes	0.239ns
(XRQ x PSC8)F3 (*Pl2*)	84 homozyg.	187 heterozyg.	63 homozyg.	1 gene	7.43*
Race 1					
(XRQ x HA335) x DF	126		31	2 genes	2.295ns
DF x (HA335 x PMI3) (DM2)	124		47	2 genes	0.563ns

lines all contain the same gene or cluster of genes. It should be noted also that they all show some proportion of plants with cotyledon limited sporulation. In contrast, a testcross of XRQ with HA335, tested with both races 1 and A gave segregation indicating that XRQ has a resistance gene or cluster independent of *Pl6*. It may be noted that since segregation was observed with race 1 and since the *Pl6* region includes *Pl2* in the strict sense, it is unlikely that XRQ contains this last gene although having resistance to races 1 and D (2 ?). The other line that can be included in the *'Pl5'* group, PMI3 (from DM2), susceptible to race A, also showed segregation in a testcross with HA335 infected with race 1. (Table 2), but crosses are not complete with the other sources of resistance from *H.tuberosus*.

The (XRQ x PSC8) F3 families tested with race A showed an apparent segregation of 84 homozygous resistant : 187 heterozygous : 63 homozygous susceptible. This does not quite agree with a Mendelian segregation of 1:2:1, but it should be noted that some plants with CLI are difficult to judge and whereas the homozygous resistant and homozygous susceptible families were retested to confirm their genotypes for the formation of bulks, this was not the case for the heterozygotes. If 4 of the 179 families judged heterozygous were in fact susceptible, the segregation would agree with a 95% probability for one gene. As with the GH x HIR34 cross, the parental lines showed polymorphism for the markers linked with the *Pl1,2,6* region (Fig.1), but the bulks did not. So far, no probe-enzyme combination has shown polymorphism between the bulks.

DISCUSSION

For *Pl4*, the two forms of HIR34 available do not differ, so this is not an explanation of the conflicting results published. The presence of CLI may be the reason for certain differences of interpretation, but the differences of results in our laboratory are certainly unexplained. It does seem most useful to study *Pl4* on its own, using bulks of plants whose genotypes present no uncertainty. Although this gene has not yet been localised, all indications are that it is not on linkage group 1.

Testcross results confirm the results of Miller and Gulya (1987)that the lines from Progress, Novinka and of Rumanian origin all contain the same gene or gene cluster, but they do suggest that *Pl5* is not in the *Pl1,2,6* region and that XRQ does not contain *Pl2* in the strict sense, but another gene giving resistance to races 1 and D (2). The molecular results go in the same direction. If *Pl5* and *Pl6* were in the same region, or if Progress contained *Pl2*, it would have been quite likely that no polymorphism would have been found between the parents for the markers such as SUN 124, but this was not the case. It seems quite possible that, for this cross, the line PSC8, has the allele of this marker linked to *Pl2* resistance, while XRQ, with *Pl5*, has the susceptible allele. What is surprising is that the bulks should show no polymorphism with the total of 180 RFLP markers tested. It may be that certain linkage groups are not well covered and further studies, including use of AFLP are in progress to find adequate markers. The resistance gene in PMI3 also appears separate from the *Pl1,2,6* regionr but crosses with lines such as XRQ and XPQ need to be completed to determine whether it is part of a '*Pl5* cluster'.

Overall, it may be questioned why both *Pl4* and *Pl5* are more difficult to locate than the genes studied earlier. One hypothesis would be that they are located in a region not covered by the markers used by Gentzbittel et al. (1995). It will important to complete both the genetic and molecular studies in progress to determine whether the '*Pl5* cluster' as suggested above really exists, with a series of genes equivalent to those in the *Pl6* cluster, giving resistance to all the French races. It would be most useful to have at least two clusters of downy mildew resistance genes which could be combined as a protection against fungal mutations leading to new races.

REFERENCES

Farrara BF, Ilott TW, Michelmore RW (1987) Genetic analysis of factors for resistance to downy mildew (*Bremia lactucae*) in species of lettuce (*Lactuca sativa* L. and *L.serriola*). Plant Pathol 36: 499-514.

Gentzbittel L, Vear F, Zhang Y-X, Bervillé A, Nicolas P (1995) Development of a consensus linkage RFLP map of cultivated sunflower (*Helianthus annuus* L.). Theor Appl Genet 90: 1079-1086 .

Leclercq P, Cauderon Y, Dauge M (1970) Sélection pour la résistance au mildiou du tournesol à partir d'hybrides topinambour x tournesol. Ann Amélior Pl 20: 363-373.

Maisonneuve B, Bellac Y, Anderson P, Michelmore RW (1994) Rapid mapping of two genes for resistance to downy mildew from *Lactuca serriola* to existing clusters of resistance genes. Theor Appl Genet 89: 96-104.

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88: 9828-9832.

Miller JF, Gulya TJ (1987) Inheritance of resistance to race 3 downy mildew in sunflower. Crop Science 27: 210-212.

Mouzeyar S. (1993) Etude de la résistance du tournesol, Helianthus annuus L., au mildiou causé par *Plasmopara halstedii*. Doctoral Thesis, Université Clermont-Ferrand,98pp.

Mouzeyar S, Tourvieille de Labrouhe D, Vear F (1993) Histopathological studies of resistance of sunflower (*Helianthus annuus* L.) to downy mildew (*Plasmopara halstedii*). J Phytopathol 139: 289-297.

Mouzeyar S, Philippon J, Walser P, Vear F, Tourvieille de Labrouhe D (1994) Sunflower resistance to French races of downy mildew (*Plasmopara halstedii*). Agronomie 14: 335-336.

Mouzeyar S, Roeckel-Drevet P, Gentzbittel L, Philippon J, Tourvieille de Labrouhe D, Vear F, Nicolas P (1995) RFLP and RAPD mapping of the sunflower *Pl1* locus for resistance to *Plasmopara halstedii* race 1. Theor Appl Genet 91: 733-737.

Roeckel-Drevet P, Gagne G, Mouzeyar S, Gentzbittel L, Philippon J, Nicolas P, Tourvieille de Labrouhe D, Vear F (1996) Colocation of downy mildew (*Plasmopara halstedii*) resistance genes in sunflower (*Helianthus annuus* L.). Euphytica 91: 225-228.

Sackston WE, Gulya TJ, Miller JF (1990) A proposed international system for designating races of *Plasmopara halstedii*. Plant Disease 74: 721-723.

Vear F. (1974) Studies on resistance to downy mildew in sunflower (*Helianthus annuus* L.). Proc.6th Int.Sunflower Conf., Bucarest, Rumania, 297-302.

Vear F, Gentzbittel L, Philippon J, Mouzeyar S, Mestries E, Roeckel-Drevet P, Tourvieille de Labrouhe D, Nicolas P (1997) The genetics of resistance to five races of downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus* L.). Theor Appl.Genet.94:584-589.

Vranceanu VL, Stoenescu FM (1970) Imunitate la mana florii soarelui conditionata monogenic. Probleme Agricole 2: 34-40

Vranceanu VL, Pirvu N, Stoenescu FM (1981) New sunflower downy mildew resistance genes and their management. Helia 4: 23-27.

Zimmer DE, Kinman ML (1972) Downy mildew resistance in cultivated sunflower and its inheritance. Crop Science 12: 749-751.