Genetical analyses
Of the sunflower Downy Mildew resistance gene *Pl5*.

Felicity Vear, **Jacqueline Phillipon**, **Sylvie Roche**, **Pascal Walser**,
Denis Tourvieille de Labrouhe, **Said Mouzeyar**, **Paul Nicolas**,
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:

A first tentative to localise the gene *Pl5*, present in the inbred line XRQ, originating from the Russian population Progress and giving resistance to French downy mildew races 100, 300,700, 703 and 710, using Bulked Segregant Analysis suggested that this gene is not in the cluster with *Pl1*, *Pl2* and *Pl6*, but no linked markers were found. In laboratory tests, this genotype gives Cotyledon Limited Infection, so that observations of resistance and susceptibilty are not always easy. Molecular studies are continuing and genetical analyses of crosses with different resistance sources have been made to provide further evidence as to the localisation and structure of this gene. XRQ was crossed with HA338 (*Pl7*), RHA340 (*Pl8*), QHP1 (*Pl?*), PMI3 (*Pl?*), YSQ (*Pl?*) and HIR34 (*Pl4*). Test crosses (susceptible x F1) using primary and secondary infections with races 100, 703 and 710 indicate that *Pl5* is independant of *Pl7* and the genes in QHP1 and PMI3 and probably Pl4, but tightly linked to *Pl8* and the gene in YSQ, perhaps forming a cluster. In all cases, the resistances to different races found in parental lines did not segregate in the test-cross progenies. Secondary infections eliminated the difficulty of interpreting plants with cotyledon limited sporulation. It was observed that progenies which showed this phenotype with primary infections, showed yellow blotches on leaves with secondary infections, but no sporulation. It is concluded that at least two linkage groups carry genes giving resistance to all the races of downy mildew known in France.

Résumé :

Une étude moléculaire mettant en oeuvre un "Bulked Segregant Analysis" n' a pas permis de localiser le gène *Pl5*, originaire de la population russe Progress et donnant la résistance aux 5 races françaises de *Plasmopara halstedii* (100, 300, 700, 703 et 710). Cependant, cette analyse semble montrer que ce gène, présent chez la lignée XRQ, n'est pas localisé dans le cluster contenant *Pl1*, *Pl2* et *Pl6*. Lors des tests sur plantule, ce génotype donne des réactions de Type II (Cotyledon Limited Sporulation). XRQ a été croisé avec HIR34 (*Pl4*), HA338 (*Pl7*), RHA340 (*Pl8*), QHP1 (*Pl?*), PMI3 (*Pl?)* et YSQ (*Pl?*). Des testcrosses (sensible x F1) utilisant des infections primaires sur racines et des infections secondaires sur feuilles ont été réalisés avec les races 100, 703 et 710. Le test réalisé sur feuilles permet de lever les incertitudes liées à la presence de sporulation sur cotylédons observées en infection primaire. Ces tests ont montré que *Pl5* n'est pas lié à *Pl7*, ni aux gènes de QHP1 et PMI3. Ce gène semble indépendant de *Pl4* mais il est lié à *Pl8* et au gène de YSQ. Ces gènes pourraient constituer un deuxième cluster situé sur un autre groupe de liaison que le cluster contenant *Pl1*, *Pl2* et *Pl6*. Nous n'avons pas observé de ségrégation entre les résistances aux différentes races apportées par les lignées parentales.

Introduction

Changes in the most widespread downy mildew (*Plasmopara halstedii*) races have been reported in recent years (Gulya, 1991), so knowledge of the race specificities, linkage group localisations and possible combinations of resistance genes is important. In France, five races (100, 300, 700, 703, 710) have been identified (Tourvieille,1999). The resistance genes at present denoted *Pl5* (from Progress (*Helianthus tuberosus* ?), INRA line XRQ), *Pl6 (*from wild *H.annuus*, USDA line HA335*)* , *Pl7 (*from *H.praecox*, USDA line HA338*)*, *Pl8* (from *H.argophyllus*, USDA line RHA340) (Miller and Gulya, 1991) and a further source of resistance provided by R. Urs (personal communication, 1984 , INRA line YSQ) give resistance to all these races. The INRA line PMI3, bred from DM2 (Novinka) gives resistance to all French races except 710, whereas the INRA line QHP1, bred from HAR5 (Charata.INTA), is resistant to all except 703.

Pl6 has been shown to be a cluster of resistance genes, located in the same area as *Pl1* and *Pl2*, and which may be separated by recombination into at least two zones, one giving resistance to races 100 and 300 and the other to races 700, 703 and 710 (Vear et al., 1997). Vear et al. (1998) reported a tentative to locate *Pl5* on the molecular map of Gentzbittel et al (1995). A test-cross between XRQ and HA335 showed segregation and *Pl5* was found not linked to the same markers as *Pl1*, *Pl2* and *Pl6*, but Bulked Segregant Analysis did not permit its location. Further molecular work is in progress, but to obtain some idea of which genes giving resistance to most or all French races are located together, (and so cannot be combined in hybrid varieties), traditional genetical studies were undertaken on test-cross progenies from crosses between XRQ and the other lines listed above and also the INRA line HIR34, which has resistance from *H.tuberosus*, but effective only against races 100 and 300.

Pl5 gives Type II resistance (Mouzeyar et al, 1993), that is, in downy mildew tests using infection of germinated seed, it shows cotyledon limited sporulation (CLS). In some conditions, it may be difficult to determine whether some seedlings are resistant or susceptible. To overcome this difficulty, secondary infection tests, on the first true leaves, were used in addition to the traditional downy mildew resistance testing method.

Materials and Methods

Sunflower genotypes:

The crosses studied are listed in Table 1. The CMS form of XRQ was used to make the F1 hybrids. Two susceptible INRA lines (FN and GB) were used to make the tests cross progenies.

Downy mildew races:

Tests were made in separate growth chambers with races 100, 703 and 710, maintained at INRA, Clermont-Ferrand.

Testing procedures:

The usual primary infection method for resistance tests followed the procedure described by Mouzeyar et al.(1993). The secondary infection procedure, on two-week old plants, was that of Meliala (1999). Observations were made 15 days after infection, after the plants had been maintained 48h in a saturated atmosphere. Plants showing no sporulation or only a light sporulation on the cotyledons and none on the true leaves were considered as resistant. For primary infections, plants with considerable sporulation on cotyledons and very small true leaves or which were damped off with no downy mildew sporulation were put in an intermediate category (denoted « ? »). Plants with sporulation on true leaves were considered as susceptible. In some cases, for secondary infections, it was necessary to use a hand lens to

observe sporulation. All observations were made without knowing the genotype in question. Each tray contained a susceptible control, which showed 100% primary or secondary infection.

Results

Results are presented in Table 1. The first tests, made in March 1999 were difficult to interpret, the proportion of apparently susceptible plants often exceeding the theoretical maximum of 25% . With race 100, damping off was frequent, and when no downy mildew spores were visible, it was not possible to tell whether the plants had been killed by this or another disease. In the case of (XRQ x YSQ), there was no sporulation, but most plants showed browning and damping off. With race 710, judgement of the first true leaves was difficult because they were often very small.

When the second series of tests was made, each tray contained half primary infections and half secondary infections (for the latter, the seeds were sown two weeks earlier, then all were infected and observed at the same time). There were fewer damping off problems for the primary infections, perhaps because of the greater space between polythene sheeting and compost. The criterion for susceptibility in the secondary infection test was sporulation on the first leaves. It was noted that progenies which showed CLS in primary infections frequently showed yellow spotting of the first leaves, but no sporulation.

The segregation of the cross XRQ x HA338 presented an apparent excess of susceptible plants in the first test with race 100, but agreed with 3R:1S for race 710 if the unreadable plants were considered as resistant. The other results for this cross agree with the 3R:1S ratio, indicating two independent genes each giving resistance to races 100, 710 and 703. In contrast, no segregation appeared in the crosses between XRQ and either RHA340 or YSQ. There were some unreadable plants in the first tests. In all cases resistance was of Type II. These results indicate that *Pl5*, *Pl8* and the gene in YSQ are the same or closely linked and all give resistance to the three downy mildew races tested. The tests with races 100 and 710 for XRQ x QHP1, 100 and 703 for XRQ x PMI3 and 100 for XRQ x HIR34 all showed some segregation. Generally, the chi square test agreed with a 3R:1S ratio when the unreadable plants were considered as resistant. If they were taken as susceptible, there were excesses of susceptible plants. The progeny from the cross XRQ x HIR34 were difficult to interpret because, quite frequently, there were a few spores on one true leaf but other downy mildew symptoms did not develop. However, even if some confirmations would be useful, the most likely interpretation of these results is that the resistance genes in QHP1, PMI3 and HIR34 are located independently of *Pl5*.

Discussion

Type II resistance gives complete resistance in the field and is used in commercial hybrids, but it can be difficult to interpret in primary seeding resistance tests, when conditions are ideal for downy mildew and seedlings not particularly vigorous. Problems encountered in Bulked Segregant Analyses to locate *Pl5* could, in part, result from difficulties in interpreting primary infection test results, although molecular marker polymorphism is probably the main cause of difficulties in location. The present study has shown the usefulness of making several series of tests with different races and of including secondary infection, which avoids the

problem of sporulation on cotyledons, to conclude on the presence of segregation in test cross progenies. It has given the first observations of type II reactions after secondary infections.

Table 1. Results of downy mildew resistance tests on test-cross progenies between XRQ (*Pl5*) and other sources of resistance (Sus.: susceptible; Res : resistant; prim : primary infection; sec : secondary infection; ? : difficult to determine if resistant or susceptible; X^2(3:1) : X^2 test to determine if segregation is in agreement with the ratio 3R:1S for two independent genes; S : plants certainly susceptible counted as susceptible, S+? : all possibly susceptible plants counted as susceptible).

Progeny	Race	Date	Test	Sus.	?	Res	Total	Comments	X^2 (3:1)	
(Sus. x (XRQ x HA338))TC	100	03/99	prim.	53	14	70	137	? : damping off	S : 13.78**	S+? : 41.8**
	710	03/99	prim.	49	17	117	183	? : very small true leaves	S : 0.31ns	S+? : 12.0**
	710	11/99	prim	21	0	84	105		S : 1.40ns	
	710	11/99	sec.	19	0	55	74		S : 0.01ns	
	703	10/99	prim.	1	7	21	29	? : damping off	S : 7.19**	S+? : 0.10ns
	703	10/99	sec.	7	0	31	38		S : 0.82ns	
(Sus. x (XRQ x RHA340)TC	100	03/99	prim.	0	5	127	132	? : very small true leaves	S : 42.4**	S+? : 31.7**
	710	03/99	prim.	0	17	233	250	? : very small true leaves	S : 83.0**	S+? : 44.6**
	710	11/99	prim	0	0	113	113		S : 37.6**	
	710	11/99	sec.	0	0	103	103		S : 34.5**	
	703	10/99	prim.	0	0	38	38		S : 12.7**	
	703	10/99	sec.	0	0	41	41		S : 13.7**	
(Sus. x (XRQ x YSQ))TC	100	03/99	prim.	0	0	156	156	R : brown spots, damping off ?	S : 52.0**	
	710	03/99	prim.	0	53	190	243	? : very small true leaves	S : 81.0**	S+? : 0.99ns
	710	11/99	prim	0	1	95	96		S : 32.0**	S+? : 29.3**
	710	11/99	sec.	0	0	75	75		S : 25.0**	
	703	10/99	prim.	0	1	37	38		S : 12.7**	S+? : 8.1**
	703	10/99	sec.	0	1	40	41		S : 13.6**	S+? : 11.1**

Table 1. continued

Progeny	Race	Date	Test	Sus.	?	Res	Total	Comments	X^2 (3:1)	
(Sus. x (XRQ x QHP1))TC	100	03/99	prim.	16	4	60	80	? : damping off	S : 1.06ns	S+? : 0ns
	710	03/99	prim.	37	23	98	158	? : very small true leaves	S : 0.21ns	S+? : 14.2**
	710	11/99	prim.	10	15	68	93	? : very small true leaves	S : 10.1**	S+? : 0.17ns
	710	11/99	sec.	32	0	65	97		S : 3.31ns	
(Sus. x (XRQ x PMI3))TC	100	03/99	prim.	65	22	139	236	? : damping off	S : 0.80ns	S+? : 17.7**
	100	12/99	prim.	16	21	53	90	? : very small true leaves	S : 2.50ns	S+? : 12.5**
	100	12/99	sec.	29	0	62	91		S : 2.29ns	
	703	10/99	prim.	0	0	41	41		S : 54.7**	
	703	10/99	sec.	8	0	33	41		S : 0.66ns	
(Sus. x (XRQ x HIR34))TC	100	03/99	prim.	50(?)	0	166	228	S? : few spores / leaves	S : 1.15ns	
	100	12/99	prim.	14(?)	51	30	95	S? : very small true leaves	S : 5.33*	S+? : 95.6**
	100	12/99	sec.	35	0	51	86		S : 11.3**	

Tests with three different races confirmed that the resistance of each of the parental lines follows the same pattern in each test, that their genes giving resistance to different races are so tightly linked that medium sized populations do not show recombination and separation of the different resistances. This study has provided useful results for breeding programmes. It confirmed that *Pl5* is in a different linkage group from the *Pl1, Pl2, Pl6* cluster, but, most interestingly, it appears to belong to the same group as *Pl8*. This was unexpected since *Pl5* is thought to come from *H.tuberosus* and *Pl8* from *H.argophylus*. YSQ had resistance of an unknown origin, and thus may be either linked, or identical, to one of these genes. It may be noted that, compared with the *Pl6* cluster, this group shows more frequent type II resistance. Location of this group on the molecular map should now be simpler as it should be easier to find sufficient polymorphism of molecular markers using any of the three resistance sources.

The three other lines used in the crosses appear to have resistance genes independant of *Pl5*. Vear et al (1998) found *Pl4* (HIR34) and the gene in PMI3 not to be in the *Pl6* cluster whereas the genes in QHP1 has not been identified and could form part of the *Pl6* cluster. It was rather unexpected that the gene in PMI3 should appear independent of *Pl5* since it was bred from DM2, a USDA pool selected from Novinka, a sister population to Progress and thought to come from the same interspecific cross with *H. tuberosus.* The downy mildew resistance of HIR34 also comes from *H.tuberosus* (Leclercq et al, 1970) but since it was a different interspecific cross, and the gene integrated to the sunflower genome possibly by a translocation, resistance could well be located on a different linkage group.

In conclusion, *Pl5* and *Pl8* and the resistance of YSQ appear very closely linked and it is confirmed that they are independent of the widely used *Pl6* cluster on linkage group 1. It will therefore be possible to develop hybrids which have dual resistance to all French downy mildew races, providing an insurance against possible race changes.

References

GENTZBITTEL L, VEAR F, ZHANG Y-X, BERVILLE A, NICOLAS P (1995) Development of a consensus linkage map of cultivated sunflower (*Helianthus annuus* L.). Theor. Appl. Genet., **90**, 1079-1086.

GULYA T.J., SACKSTON W.E., VIRANYI F., MASIREVIC S., RASHID K.Y. 1991.New races of sunflower downy milew pathogen (*Plasmopara halstedii*) in Europe and North and South America. J.Phyopathology, 132:303-311.

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.

MELIALA C. VEAR F., TOURVIEILLE de LABROUHE D. 1999 Relations between date of infection of infection of sunflower downy mildew (*Plasmopara halstedii)* and symptom development. Helia (in press).

MILLER J, GULYA T (1991) Inheritance of resistance to race 4 of downy mildew derived from interspecific crosses in sunflower. Crop Sci. 31:40.43.

MOUZEYAR S., TOURVIEILLE de LABROUHE D., VEAR F., 1993. Histopathological studies of resistance of sunflower (*Helianthus annuus* L.) to downy mildew (*Plasmopara halstedii* Farl. Berlese et de Toni). J. Phytopathology, **139**, 289-297

TOURVIEILLE de LABROUHE D. 1999 La nouvelle nomenclature des races de *Plasmopara halstedii*, agent du mildiou du tournsol, appliquée aux races françaises. OCL, 6: 219-222..

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., **95**, 584-589.

VEAR, F., MILLON J., PHILIPPON J., MOUZEYAR S., NICOLAS P., TOURVIEILLE de LABROUHE. 1998 Analysis and location of sunflower downy mildew resistance genes. In ed. GULYA T, VEAR F. 1998. I.S.A. symposium « Sunflower Downy Mildew ». Fargo, USA 13-14/01/1998. 84-93.