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INHERITANCE OF THE WIDE-RANGE DOWNY MILDEW RESISTANCE IN THE SUNFLOWER LINE RHA 419

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SUMMARY

Downy mildew resistance in the USDA inbred sunflower line RHA 419 was studied in F_2 and test cross progenies from crosses with a susceptible line and with lines carrying known resistance genes. RHA 419 gives resistance to all races known at present, of type II, with frequent sporulation on cotyledons in seedling tests. The cross with a susceptible line indicated that one dominant gene imparted resistance to races 304 and 710. The progenies from crosses with lines carrying *Pl5, Pl6* and *Pl8* all showed segregation when tested with races 304, 710 and 730,generally agreeing with the hypothesis of two independent genes. It was concluded that RHA 419 provides a diversification of resistance and its use in breeding is discussed.

Key words: ***Plasmopara halstedii*, disease race, *Helianthus annuus*, test cross, durability, inheritance**

INTRODUCTION

Downy mildew (*Plasmopara halstedii*) a world-wide disease of sunflower, with at least 15 races now reported (Viranyi, 2002; Tourvieille de Labrouhe *et al.*, 2003). Up to the present, it has been controlled, quite successfully, by major genes, denoted *Pl* (Vranceanu and Stoenescu, 1970; Miler and Gulya, 1991; Vear *et al.*, 2000). Several of these genes have been found to be grouped into clusters, *Pl1, Pl2, Pl6* and *Pl7* were mapped as Group I (Roeckel-Drevet *et al.*, 1996) of the Cartisol map (Gentzbittel *et al.*, 1995), which is group 8 of the map of Yu *et al.*, 2003. More recently, *Pl5* and *Pl8* have been mapped as group 6 (Bert *et al.*, 2001; Radwan *et al.*, 2002) of the Cartisol map (group 13 of Yu *et al.*, 2003).

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Following what is known for other crop-pathogen interactions (Ye *et al.*, 2002), durability of downy mildew resistance would most usefully combine as wide a range of *Pl* genes as possible (Penaud *et al.*, 2000). For this reason, a search has been made for new *Pl* genes, in particular in wild *Helianthus* species. Miller *et al.* (2002) reported the registration of a restorer line, RHA 419, developed from the cross (RHA 373 × ARG 1575-2). ARG 1575-2 was derived from the cross *cms* HA 89 × *H.argophyllus* accession Raf.-1575 (PI468651). RHA 419 obtained its downy mildew resistance from *H.argophyllus* and is resistant to American races 300, 700, 730 and 770. This paper reports studies on the genetics of this resistance, in the USA and France, using several races of downy mildew.

MATERIAL AND METHODS

Sunflower lines

RHA 419 was crossed with HA 335 (*Pl6*), HA 338 (*Pl7*) and RHA 340 (*Pl8*) (Miller and Gulya, 1991) by USDA, Fargo, USA, and with a completely susceptible line and with YDQ (*Pl6*) and XRQ (*Pl5*) (Vera *et al.*, 2000) by INRA, Clermont-Ferrand, France. The F_1 plants were either selfed to give F_2 progenies or crossed with completely susceptible inbred lines to give test cross progenies.

Downy mildew tests

Seedling immersion test were used following the methodologies of Gulya *et al.* (1991) and Mouzeyar *et al.* (1993). In the USA, tests were made with race 730. In France, tests of the inbred line were made with all the races available at INRA Clermont-Ferrand and inheritance studies with races 304 and 710. Plants showing some sporulation on cotyledons but continuing to grow satisfactorily were considered to show the "Type II" resistance well known for *Pl5* and *Pl8*, for example (Bert *et al.*, 2001).

RESULTS

RHA 419 was observed to be resistant to French races 100, 300, 304, 307, 314, 700, 704, 710 and 714 and to a sample of the USA race 330, in addition to those reported by Miller *et al.* (2002).

Table 1 presents the results of the resistance tests on segregating progenies, with several replications for each race. With races 304 and 710, type II resistance was frequent and in some replications there were plants that were difficult to judge because they did not have sporulation on leaves but grew very weakly (type IIII). They were considered as susceptible but this may not always have been the case.

The cross with the completely susceptible line gave a test cross progeny showing segregation of 1R:1S with race 710, indicating that one gene controls resistance

to this race. One of the replications showed an excess of susceptible plants compared with the other replicates, probably because there was quite a large number of plants (15) showing type III reaction. Overall, the X^2 was not significantly different from 1R:1S.

Table 1: Results of downy mildew tests on test cross and F_2 progenies between RHA 419 and other sources of downy mildew resistance

		Resistant	Susceptible	Total	X^2		
a) Race 710							
susceptible × (susceptible × RHA 419) F_1		46	57	103	1:1	1.17	ns
		47	53	100	1:1	0.36	ns
		29	59	88	1:1	10.2	**
	total	122	169	291	1:1	3.80	ns
susceptible × (YDQ × RHA 419) F_1		72	12	84	3:1	5.15	*
		74	18	92	3:1	1.45	ns
		80	17	97	3:1	2.88	ns
	total	226	47	273	3:1	8.82	**
susceptible × (XRQ × RHA 419) F_1		83	16	99	3:1	4.12	*
		81	22	103	3:1	0.83	ns
		78	20	98	3:1	1.01	ns
	total	242	58	300	3:1	5.03	*
b) Race 304							
susceptible × (YDQ × RHA 419) F_1		70	21	91	3:1	0.18	ns
		56	13	69	3:1	1.38	ns
		77	24	101	3:1	0.09	ns
	total	203	58	261	3:1	1.07	ns
susceptible × (XRQ × RHA 419) F_1		61	19	80	3:1	0.01	ns
		62	21	83	3:1	0.00	ns
		79	28	107	3:1	0.08	ns
	total	202	68	270	3:1	0.01	ns
c) Race 730							
(HA 335 × RHA 419) F_2		105	19	124	15:1	17.41	**
(HA 338 × RHA 419) F_2		104	10	114	15:1	1.24	ns
(HA 419 × RHA 340) F_2		110	20	130	15:1	8.51	**

In all the progenies from the crosses between RHA 419 and the other sources of downy mildew resistance, there was segregation for resistance. With races 304 and 710 there was, for each test cross, one replication with an excess of resistant plants, but generally the segregation ratios agreed with a ratio of 3R:1S, showing the presence of two independent genes. In contrast, two of the F_2 progenies tested with race 730 showed more than 1/16 of plants susceptible (crosses with HA 335 and RHA 340), but this may be because all plants showing sporulation were considered as susceptible. These segregations did not agree with complete resistance which would

be case if the genes were closely linked. Therefore, it was concluded that, in these crosses, two independent genes were also imparting resistance to race 730.

Overall, these results indicate that RHA 419 carries a gene/cluster for resistance to many downy mildew races which is independent of the *Pl6*/*Pl7* and *Pl5*/*Pl8* clusters.

DISCUSSION

The results reported here indicate that several different *Pl* genes can be obtained from *H.argophyllus*, since *Pl8* was also obtained from that species (Miller and Gulya, 1991). This is encouraging since it suggests that further new *Pl* genes may be found. The gene/cluster in RHA 419 has not yet been mapped and it remains to be determined whether its structure is different from those analyzed (Radwan *et al.*, 2002).

It has already been reported that such "genes" giving resistance to a large number of races, for example *Pl5*, resistant to all French races, and *Pl8*, resistant to all known races, show sporulation on cotyledons (Bert *et al.*, 2001; Radwan *et al.*, 2002). These results concerning RHA 419 confirm that interesting "strong genes" may show resistance that takes some time to be effective in the plant, but is quite sufficient in the field. It does mean that inheritance studies using seedling tests may be complicated by difficulties in judging all plants and that replications over several tests and comparisons between different races and sunflower genotypes are necessary to make conclusions about the control of resistance.

A recent study of the evolution of races in France showed that varieties containing *Pl1* and *Pl2* gave resistance to race 100 for more than 10 years (1978-1988), and the use of *Pl6* and *Pl7* to control races 710 and 703 which appeared in 1988 and 1989 (Lafon *et al.*, 2000) was effective until the discovery of race 304 in 2000 (Tourvieille de Labrouhe *et al.*, 2000), which is again about 10 years. Recently, the area of sunflower hybrids containing *Pl5* and *Pl8* has become significant in France and if these resistances are used on their own, it may be hypothesized that they will probably be overcome by 2010 (Tourvieille de Labrouhe *et al.*, 2003).

The gene in RHA 419 will thus be very useful in breeding but its best contribution to durability of resistance needs to be considered carefully. The fact than, so far, this resistance seems to be effective against all known races of downy mildew means that combining with other genes/clusters in one inbred line will be difficult to determine and will require the use of molecular markers. However, the fact that sunflowers are a hybrid crop means that resistance in the restorer line RHA 419 can be combined with *Pl5* and *Pl6* in a female line to create a hybrid with multiple resistance. Knowledge of the gene structure would make it possible top determine if different mutations in the pathogen would be necessary to overcome this multiple resistance. For example it is important to know whether it is not only the location of this gene/cluster which is different from *Pl8* but also its mechanism of activity.

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REFERENCES

Bert, P-F., Tourvieille de Labrouhe, D., Phillippon, J., Mouzeyar, S., Jouan, I., Nicolas, P. and Vear, F., 2001. Identification of a second linkage group carrying genes controlling resistance to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. 103: 992-997.

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

Gulya, T.J., Miller, J.F., Viranyi, F. and Sackston, W.E., 1991. Proposed internationally standardized methods for race identification of *Plasmopara halstedii*. Helia 14(15): 11-20.

Lafon, S., Delos, M., Raulic, I. and Tourvieille de Labrouhe, D., 2000. Bilan de dix années de surveillance du mildiou du tournesol en France. 15th Int. Sunflower Conf. Toulouse, France, 12-15. 06. 2000, pp. I49-I54.

Miller, J.F. and Gulya, T.J., 1991. Inheritance of resistance to race 4 of downy mildew derived from intespecific crosses in sunflower. Crop Sci. 31: 40-43.

Miller, J.F. and Gulya, T.J. and Seiler, G.J., 2002. Registration of five fertility restorer sunflower germplasms. Crop Sci. 42: 989-991.

Mouzeyar, S., Tourvieille de Labrouhe, D. and Vear, F., 1993. Histopathological studies of resistance of sunflower (*Helianthus annuus* L.) to dawny mildew (*Plasmopara halstedii* Farl. Berlese et de Toni). J. Phytopathology 139: 289-297.

Penaud, A., Roeckel-Drevet, P., Meliala, C., Pilorgé, E. and Tourvieille, J., 2000. Evolution de la maladie, du parasite et des methods de lutte. In: "Le mildiou du tournesol", Tourvieille de Labrouhe, D., Pilorgé, E., Nicolas, P. and Vear, F. (ed.) INRA, pp. 145-149.

Radwan, O., Bouzidi, M-F., Vear, F., Philippon, J., Tourvieille de Labrouhe, D., Nicolas, P. and Mouzeyar, S., 2002. Identification of non-TIR-NBS-LRR markers linked to the *Pl5/Pl8* locus for resistance to downy mildew in sunflower. Theor. Appl. Genet. 106: 1438-1446.

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

Tourvieille de Labrouhe, D., Lafon, S., Walser, P. and Rulic, I., 2000. Une nouvelle race de *Plasmopara halstedii*, agent du mildiou du tournesol. O.C.L. 7: 404-405.

Tourvieille de Labrouhe, D., Batailon, C., Moinarad, J., Philippon, J., Walser, P., Tourvieille, J. and Vear, F., 2003. Evolution of downy mildew races in France. In preparation.

Vear, F., Gentzbittel, L., Philippon, J., Mestries, E., Roeckel-Drevet, P., Tourvieille de Labrouhe, D. and Nicolas, P., 1997. The genetics of resistance of five races of downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. 95: 584-589.

Vear, F., Philippon, J., Roche, S., Walser, P., Tourvieille de Labrouhe, D., Mouzeyar, S., and Nicolas, P., 2000. Genetical analyses of the sunflower downy mildew resistance gene *Pl5*. Proc. of 15th Int. Sun. Conf., Toulouse, France, 12-16.06.2000, pp. J31-J36.

Viranyi, F., 2002. Downy mildew research in sunflower: Facts and consideration (based on reports of contributors to the FAO sub-group, *Plasmopara halstedii* for 1999-2001). FAO Technical Meeting, 7-9.10.2002, ENSAM-INRA, Montpellier, France.

Vranceanu, V and Stoenescu, F., 1970. Immunity to sunflower downy mildew due to a single dominant gene. Probleme Agric. 22: 34-40.

Ye, G., McNeil, D.L. and Hill, G.D., 2002. Breeding for resistance to lentil *Ascochyta* blight. Plant Breeding 121: 185-191.

Yu, J.K., Tang, S., Slabaugh, M.B., Heesacker, A., Cole, G., Herring, M.J., Soper, J., Han, F., Chu, W.C., Webb, D.M., Thompson, L., Edwards, K.J., Berry, S., Leon, A., Olungu, C., Maes, N. and Knapp, S.J., 2003. Towards a saturated molecular genetic linkage map for cultivated sunflower. Crop. Sci. 42: 367-387.

HERENCIA DE AMPLIA RESISTENCIA A TIZÓN EN LA LÍNEA DE GIRASOL RHA 419

RESUMEN

En el trabajo se investigaba la resistencia de la línea inbred de girasol RHA 419 USDA, a tizón. Las investigaciones se hicieron en la descendencia de la generación F_2 y prueba de cruzamiento, obtenido por combinación de una línea sensible con las líneas que contienen los genes de resistencia conocidos. La línea RHA 419 proporciona la resistencia a todas las razas de este patógeno, conocidas hasta el momento (tipo II) con una frecuente esporulación en cotiledones en las pruebas de plántulas. El cruzamiento con la línea sensible demostró que la resistencia a razas 304 y 710, la proporciona el gen dominante. En todas las descendencias del cruzamiento con las líneas que poseen los genes *Pl5, Pl6, Pl7* ó *Pl8*, está determinada la segregación en ejercicio de pruebas con las razas 304, 710 y 730, lo que generalmente coincide con la hipótesis de dos genes independientes. Se ha deducido que la línea RHA 419 posibilita la extensión de resistencia, y se está considerando su utilización en la mejora genética.

TRANSMISSION D'UNE GRANDE RÉSISTANCE AU MILDIOU DANS LA LIGNE DE TOURNESOL RHA 419

RÉSUMÉ

Cette étude porte sur la résistance au mildiou de la ligne inbred de tournesol RHA 419 (USDA). Les recherches portaient sur les descendants de la génération F_2 et le test de croisement des descendants obtenus avec une ligne sensible et des lignes portant des gènes de résistance connus. La ligne RHA 419 offre une résistance à toute les races de ce pathogène (Type II) connues jusqu'à présent avec fréquente sporulation sur les cotylédons des pousses. Le croisement avec une ligne sensible a montré que la résistance à la race 304 et 710 est donnée par un gène dominant. Lors des tests avec les races 304, 710 et 730, une ségrégation a été établie dans tous les descendants du croisement avec les lignes portant les gènes *Pl5*, *Pl6*, *Pl7* ou *Pl8*, ce qui corrobore l'hypothèse de deux gènes indépendants. En conclusion, on peut dire que la ligne RHA 419 diversifie la résistance et on considère son utilisation dans la culture.