NEW SUNFLOWER DOWNY MILDEW RESISTANCE GENES AND THEIR MANAGEMENT

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INTRODUCTION

Downy mildew incited by the fungus *Plasmopara helianthi* Novot. has constituted one of the most destructive diseases of sunflower in Europe, since high open pollinated varieties have been introduced and extended in commercial production.

The first genetic resistance source was identified by Vrânceanu (1967) in the Romanian inbred line AD-66 and described as a type of specific, vertical resistance, controlled by the major dominant gene Pl_1 (Vrânceanu, 1970; Vrânceanu and Stoenescu, 1970). This gene conditions resistance to the European race of *P. helianthi*, but is ineffective against the more virulent North American race. Resistance to the latter race was communicated by Zimmer and Kinman (1971) as conditioned by Pl_2 gene existing in the branched line HA-61.

On these bases, the first downy mildew resistant hybrids have been developed, permitting to control almost completely the *P. helianthi* attack. Nevertheless, assuming that the hostparasite interaction is a permanent and keen struggle for survival between two organisms, our investigations, exposed in this paper, have further followed the evolution of the competition between sunflower and Plasmopara, for being able to intervene in time in favour of the maintenance of the host genetic resistance as long as possible.

MATERIALS AND METHODS

The investigations were performed at Fundulea, Romania, under phytotron and greenhouse controlled environment and in a heavy infested field testing nursery. A great number of genotypes of local or foreign origin was screened, as well as the current breeding material used to select resistant inbred lines.

The following sunflower inbreds and hybrids were used to detect new Plasmopara races: AD-66 (Pl_1Pl_1), RHA-274 (Pl_2Pl_2), HA-61 ($Pl_2Pl_2Pl_3Pl_3$), S-1358 (susceptible inbred). Six new downy mildew resistant inbreds obtained at Fundulea were included in a diallel cross system along with AD-66 and HA-61, in order to establish the allelism of their *Pl* genes.

The artificial inoculations were performed with zoosporangia collected from infected plants previously kept for sporulation in humid chambers at 18°C. The germinated seeds were maintained in a suspension of 10,000—15,000 zoosporangia per ml in distilled water, at 18—20°C for 6—8 hours. The inoculated seeds were planted in boxes with a mixture of sterile soil and sand. Determinations were performed after 12 days, in the stage of the first pair of true leaves, after the seedlings were kept at 18°C and 95% air moisture for 24 hours, in order to induce the fungus sporulation.

RESULTS AND DISCUSSION

A break in genetic resistance of AD-66, known as differential line for the European race of *P. helianthi*, was disclosed in 1975 in Fundulea testing nursey heavily infected by downy mildew, due to a long-term sunflower monoculture. This sensitivization consisted in the appearance of a very reduced percentage of diseased plants within that line, but the attack has increased gradually from year to year reaching 26% in 1981 (Table 1).

The reaction of the differential lines AD-66 (Pl_1Pl_1), HA-61 ($Pl_2Pl_2Pl_3Pl_3$) and S-1358 (susceptible) evidenced the existence of a new, more virulent downy mildew race, designated "Fundulea race". The new race attacks completely the inbred line AD-66 and the other genotypes containing the Pl_1 gene, but does not infect inbreds carrying the Pl_2 gene. It has been assumed that Fundulea race appeared as a consequence of the selection pressure put on the pathogen, the testing nursery being overloaded with downy mildew resistant sunflowers (Vrânceanu et al., 1978).

Table 1

Reaction of four sunflower differential lines to different isolates of *Plasmopara helianthi* Novot. (artificial infections in phytotron, 1981)

Source of inoculum (Location-District)	Frequency of diseased plants (%) *			
	AD-66 Pl_1Pl_1	RHA-274 Pl_2Pl_2	HA-61 Pl_2Pl_2 Pl_3Pl_3	S-1358 Check for susceptibility
C.A.P. Dor Mărunt, Ialomiţa	0	0	0	100
S.C.A. Mărculeşti, Ialomiţa	0	0	0	98
S.C.A. Caracal, Olt	0	0	0	100
S.C.A. Lovrin, Timiş	0	0	0	99
S.C.A.Z. Secuieni, Neamţ	0	0	0	97
S.C.A. Podu Iloaie, Iaşi	0	0	0	94
C.A.P. Fundulea, Călăraşi	0	0	0	98
I.C.C.P.T. Fundulea (downy mildew nursery)	26	0	0	98
I.C.C.P.T. Fundulea (phytotron, inoculum from infected plants of AD-66)	100	0	0	100

* The number of tested plants ranged from 126 to 185.

At present, Fundulea race is isolated in phytotron and used for artificial inoculations in order to develop resistant genotypes, capable to overcome the virulence of the new race when it will spread in commercial production fields. The expansion of the new race is surveyed annually by collecting inoculum from the main sunflower growing areas and infecting the differential sunflower set with each source of inoculum. Data presented in Table 1 show that the new Fundulea race has not yet spread in commercial fields, being further confined to Fundulea downy mildew testing nursery.

As shown in Table 2, five out of six downy mildew resistant inbreds developed at Fundulea and used in this study, contain *Pl* genes that condition resistance to both races. Four inbreds (prefix S_{11}) trace to a source-population based on some crosses between *H. tuberosus* and the cultivated sunflower, one inbred (RF-6940-2-3) was developed from a composite-cross population released at College Station, Texas (Z i m m e r and K i n m a n, 1971), and one inbred (CG-78—272), resistant only to the European race, was obtained by selfing and selection within an interspecific hybrid between wild annual sunflower *(H. annuus* ssp. *annuus)* and the cultivated sunflower.

The inheritance studies based on crosses of these resistant inbred lines to the susceptible inbred P-1380, using only inoculum from the European Plasmopara race, have resulted in statistically confirmed segregation ratios of 3 : 1 and 15 : 1, indicating the presence of one or two independent major genes, as follows (Table 3) :

Table 2

Reaction of nine sunflower inbred lines of different genetic background to two races of *Plasmopara helianthi* Novot. (Fundulea, 1978)

Inbreds	Origin	European race			Fundulea race		
		no. tested plants	diseased plants		no. tested plants	diseased plants	
			no.	%		no.	%
AD-66	hybrid Advent	62	0	0	32	32	100
HA-61	composite-cross population	38	0	0	41	0	0
RF-S_{11}5566-74	source-population Synthetic 11	84	0	0	56	0	0
RF-S_{11}-15-24-5-4	—„—	51	0	0	24	0	0
RF-S_{11}-6278	—„—	63	0	0	39	0	0
RF-S_{11}-6689	—„—	42	0	0	54	0	0
RF-6940-2-3	composite-cross population	18	0	0	37	0	0
CG-78-272	*H. annuus* ssp. *annuus* (Wild) × cultivated *H. annuus*	29	0	0	41	41	100
P-1380 (susc. check)	Peredovik	46	46	100	53	53	100

Table 3

Segregation ratios of F_2 and testcross populations of crosses between eight downy mildew resistant inbreds and the susceptible inbred P-1380 (artificial inoculations with the European race, Fundulea, 1979)

Inbreds	Generations	Segregation ratios (resistant : susceptible plants)		χ^2	P%
		recorded	expected		
AD-66	F_1	32 : 0	—	—	—
	F_2	72 : 24	3 : 1	0.158	0.50—0.70
	test-cross	—	—	—	—
RHA-274	F_1	35 : 0	—	—	—
	F_2	31 : 9	3 : 1	0.133	0.70—0.90
	test-cross	42 : 46	1 : 1	0.188	0.50—0.70
RF-S_{11}-5566-74	F_1	61 : 0	—	—	—
	F_2	26 : 9	3 : 1	0.009	> 0.95
	test-cross	45 : 41	1 : 1	0.186	0.50—0.70
RF-S_{11}-15-24-5-4	F_1	49 : 0	—	—	—
	F_2	53 : 3	15 : 1	0.076	0.70—0.90
	test-cross	31 : 9	3 : 1	0.133	0.70—0.90
RF-S_{11}-6278	F_1	35 : 1	—	—	—
	F_2	78 : 6	15 : 1	0.114	0,70—0.90
	test-cross	38 : 13	3 : 1	0.009	> 0.95
RF-S_{11}-6689	F_1	42 : 0	—	—	—
	F_2	135 : 10	15 : 1	0.103	0.70—0.90
	test-cross	112 : 43	3 : 1	0.621	0.30—0.50
RF-6940-2-3	F_1	24 : 0	—	—	—
	F_2	27 : 6	3 : 1	0.818	0.30—0.50
	test-cross	22 : 16	1 : 1	0.947	0.30—0.50
CG-78-272	F_1	38 : 2	—	—	—
	F_2	23 : 7	3 : 1	0.044	0.70—0.90
	test-cross	18 : 15	1 : 1	0.272	0.50—0.70

— one dominant gene : AD-66, HA-61, RF-S_{11}-5566-74, RF-6940-2-3, CG-78-272.

— two different dominant genes : RF-S_{11}-15-24-5-4, RF-S_{11}-6278, RF-S_{11}-6689.

The eight inbred lines whose resistance is conditioned by one or two independent dominant genes were introduced in a diallel cross system in view of the allelic evaluation of *Pl* genes (Table 4). Data recorded after testing the resistance to the European race in F_2 and test-cross populations showed that :

— AD-66 and CG-78-272 possess the same gene Pl_1 ;

— HA-61 and RF-6940-2-3 share the common gene Pl_2 ;

— RF-S_{11}-15-24-5-4, RF-S_{11}-6278 and RF-S_{11}-6689 are homozygous for both genes Pl_1 and Pl_2 ;

— the resistance of the inbred line RF-S_{11}-5566-74 is conferred by another gene that differs from Pl_1 and Pl_2. Because two aditional genes Pl_3 and Pl_4 had been described by Vear (1974), although Zimmer and Hoes (1978) reported that Pl_2 and Pl_4 are identical, we propose the designation Pl_5 for the new gene identified in RF-S_{11}-5566-74.

The gene Pl_5 provides resistance against all Plasmopara races known to date. Recently, F i c k and A u w a t e r (1981) have reported the appearance of a new race in the USA, ascertaining that all the existing resistance sources have become ineffective. The only resistant genotypes proved to be the Romanian sunflower hybrids having in common the inbred line RF-S_{11}-5566-74, the gene Pl_5 being thus highly effective against the new North American race (M i l l e r, 1981, personal communication).

The existence of different Plasmopara races, as well as the virulence changes occurring in the pathogen populations, imposes the adoption of a particular strategy for the development and introduction in commercial production of sunflower downy mildew resistant hybrids.

According to the gene-for-gene concept, the extension and generalization of downy mildew resistant hybrids on a large scale will intensify the selection pressure on the pathogen which will develop new races capable to overcome the host resistance. This selection is greatest when a single gene for specific resistance is widely used, as for instance in the case of the interaction between sunflower Pl_2 genotypes and Plasmopara Red River race in North America, which resulted in a new virulence of the pathogen.

In Europe, no significant changes in *Helianthus-Plasmopara* interaction have been reported, except that described in Romania but confined to downy mildew testing nursery of Fundulea. The expansion rate of Fundulea race seems to be quite low (V r â n c e a n u

Table 4

Segregation ratios (resistant : diseased plants) of F_2 and testcross populations of a diallel cross among eight downy mildew resistant inbreds (artificial inoculations with the European race, Fundulea, 1980)

Resistant inbreds	RHA-274		RF-S_{11}-5566-74		RF-S_{11}-15-24-5-4		RF-S_{11}6278		RF-S_{11}-6689		RF-6940-2-3		CG-78-272	
	F_2	test-cross	F_2	test-cross	F_2	test-cross	F_2	test-cross	F_2	test-cross	F_2	test-cross	F_2	test-cross
AD-66	128:11 15:1	47 : 19 3 : 1	80:8 15:1	50 : 16 3 : 1	93 : 0	136:0	18 : 0	86 : 4*	45 : 0	116:1*			25:0	45:0
RHA-274			179:18 15:1		101 : 0		120 : 0				16:0	92:2*		
RF-S_{11}-5566-74					130 : 1 63 : 1	100:12 7:1	72 : 0 63 : 1	165:25 7:1			126:11 15:1	35:10 3:1		
RF-S_{11}-15-24-5-4							72 : 0		91 : 0					
RF-S_{11}-6278														
RF-S_{11}-6689											108:0	39:1*	74:0	76:2*
RF-6940-2-3													60:8 15:1	81:30 3:1

* The small number of diseased plants recorded in testcross could be attributed to selfings within the susceptible female parent.

et al., 1978) and this may be attributed, among other special features of the pathogen, to its preponderant soil-borne nature. According to Robinson's rules (Robinson, 1971), this type of specific resistance could be much more effective in protecting sunflower against downy mildew than it has been the case with other diseases where specific resistance proved to be of little value.

Considering the still low number of *Pl* genes available, sunflower breeders haven't much option regarding the gene deployment methods as indicated by Frey et al., (1973). So, the introduction of *Pl* genes in units of two or three in each F_1 hybrid will provide a higher downy mildew resistance but the cultivation of such hybrids could lead to the rapid development of some extremely, virulent forms which will be difficult to control with the existing genes. Apparently, the stronger the resistance in the host, the quicker the shift in the pathogen population will be. From this point of view, it is to be expected that new more virulent Plasmopara races will appear more rapidly in those European countries that grow U.S. hybrids or other hybrids containing the Pl_2 gene than in countries growing Pl_1 hybrids, as for instance Romania.

On these grounds, in the present stage of our sunflower breeding programme, only F_1 hybrids containing Pl_1 gene are released for large-scale cultivation, the resistance gene being incorporated in the male restorer parent. At the same time, isogenic lines containing separately the genes Pl_2 and Pl_5 are developed and kept in reserve for the next stages when new virulences of the fungus could arise. In this manner, the commercial hybrids could be converted immediately, in the process of hybrid seed production, into hybrids resistant to the new physiological races, by substituting the isogenic parental lines.

A possibility to increase the duration of the monogenic specific resistance to a certain race of the pathogen is the development of the F_1 hybrids homozigous for the respective *Pl* gene, considering such forms with enriched gene dosage more efficient than the heterozygous forms. For getting such hybrids, the gene *Pl* should be incorporated not only in the genotype of the male parent, but also in that of the female pollen sterile line, a more complicated but feasible procedure which consists in transferring the *Pl* gene into the genotype of the male fertile analogue B in a first stage and then, by continuous backcrossing, into the male sterile analogue A.

CONCLUSIONS

Significant changes in *Helianthus annuus-Plasmopara helianthi* interaction have occurred in a relatively short period of time, confirming the well-known genetic considerations on host-parasite interaction. The appearance and expansion of the new races of the fungus seems however to be lower than in the case of other parasites, permitting to forsee a longer duration and a higher efficiency of sunflower specific, vertical resistance described in this paper.

Although a small number of resistance genes have been identified by now, the noteworthy frequency of such genes in the genotypes of sunflower wild species, particularly in *Helianthus tuberosus* L., creates the premises

of a permanent genetic advance of the host in its competition with the fungus. Thus, the gene Pl_5 recently identified and reported in this paper is a strong gene which cover a large array of Plasmopara races. In order to avoid the premature exhaustion of the existing reserve of *Pl* genes, they should be used carefully by their gradual incorporation in sunflower commercial hybrids, according to the appearance and spreading of new virulences of the fungus.

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NOUVEAUX GÈNES DE RÉSISTANCE AU MILDIOU DE TOURNESOL ET LA STRATÉGIE DE LEUR UTILISATION

Résumé

En 1972 on a signalé l'existence de deux races de Plasmopara helianthi Novot. : la race „européene" controlée par les deux gènes connus Pl_1 et Pl_2 et la race américaine „Red River" qui n'est controlée que par le gène Pl_2. L'apparition d'une nouvelle race („Fundulea") plus virulente que l'ancienne race européenne a été rapportée en 1975 en Roumanie, cependant qu'en 1981 une nouvelle race bien plus virulente que „Red River" est apparue au nord des Etats Unis.

La découverte dans le genotype de la lignée consanguine roumaine RF-S_{11}-5566-74 d'un nouvel gène de résistance vis-à-vis du mildiou forme l'objet du présent rapport. Jusqu'à présent on n'avait annoncé que quatre gènes Pl et, donc, pour ce nouvel gène on propose le symbole Pl_5. Le gène Pl_5 a eu une efficacité totale vis-à-vis des quatre races de mildiou connues. En tenant compte des théories modernes sur les relations plant hôte — parasite, on suggère une stratégie d'utilisation des gènes Pl pour pouvoir lutter par voie génétique le plus long temps possible contre les attaques de ce champignon.

NUEVOS GENES DE RESISTENCIA AL MILDÉN DE GIRASOL Y LA ESTRATEGIA DE SU EMPLEO

Resúmen

En 1972 se comunicó la existencia de dos razas de Plasmopara helianthi Novot : la raza „europea" contra la cual tienen eficacidad los dos genes conocidos Pl_1 y Pl_2 y la raza norte-americana „Red-River" controlada sólo por el gene Pl_2. En 1975 fue anunciada en Rumania la aparición de una nueva raza („Fundulea") más virulenta que la antigua raza europea. En 1981, en el Norte de los Estados Unidos apareció otra raza de una virulencia mayor que la „Red-River".

En el presente trabajo se comunica el descubrimiento de un nuevo gene de resistencia al mildén en el genotipo de la línea cosanguinizada rumana RF-S_{11}-5566-74. Hasta el presente siendo reportados cuatro genes *Pl*, para este nuevo gene se ha propuesto el símbolo Pl_5. El gene Pl_5 ha mostrado una eficacidad total contra las cuatro razas de mildén concidas. Tomando en consideración la teoría moderna con respecto a las relaciones anfitrión-parásito se propone una cierta estrategia en el empleo de los genes *Pl*, para lograr combatir el ataque de este parásito por vía genética, por un periodo más largo de tiempo.