

Allelic Relationship of Resistant Genes to Sunflower Downy Mildew Race 1 in Various Sunflower Inbred Lines

Muhammad Rahim

Agricultural Research Station(North), P.O. Box 22 Saidu Sharif, District Swat, N.W.F.P., Pakistan.

Abstract: F₂ and test crosses of the inbred lines with the recessive parent HA 821 were produced, tested for resistance reaction and allelic relationship of the genes; as with 3R : 1S in F₂ and IR : IS in test cross and 15R : 1S in F₂ and 3R : 1S in test cross with one gene and two genes segregation, respectively. Digenic and independent gene reaction of resistance was found in AMES 3235, PI 497250, and RHA 274, each having *PI1* and *PI12* for resistance to SDM race 1, *PI2* and *PI11* conferring resistance to race 2. DM-2 and PI 497938 each has *PI2* and RHA 266 has only *PI1* to race. 1.

Key words: *Helianthus Annuus*; *Plasmopara halstedii* Race 1; Gene Allelic relationship

Introduction

Downy mildew incited by *Plasmopara halstedii* (Farl.) Berl & de Toni, is considered one of the most serious diseases of sunflower (*Helianthus annuus* L.) (Zimmer and Hoes, 1978). It is a destructive soil and air borne pathogen of sunflower (Zimmer, 1971). The disease is characterized by distinctive systemic and localized phases. Localized infection occurs primarily on leaves and seldom gives rise to systemic symptoms (Zimmer, 1975). Systemic infection can reduce yield up to 50% (Zimmer, 1971). With the introduction of high oil sunflower cvs. into Europe the downy mildew pathogen became one of the limiting factors of sunflower production throughout the continent (Viranyi, 1984). The increase in sunflower acreage in the Red River valley of US has been accompanied by increased prevalence of downy mildew. The heavy clay soil and flat topography of the Red River area results in poor water drainage, which favours downy mildew (Zimmer, 1971). Sunflower downy mildew originated in North America (Leppik, 1966) and spread throughout important growing regions world wide. The disease reduces both yield and oil quality (Zimmer and Zimmerman, 1972). Resistance to *P. halstedii* in cultivated sunflower has been traced to wild *H. annuus* L., other helianthus species and cultivated sunflower germplasm lines (Sackston, 1981).

The first sunflower variety "INRA 7702", resistant to SDM was registered in 1970, and the second variety "Remil", was registered in 1974 in France (Vear, 1974). Physiological races of *P. halstedii* were first reported by (Zimmer, 1974). In 1991 a total of eight races of sunflower SDM were reported in North America. Races 1, 4 and 6 were confined in Europe and races 2, 3, and 4 in Asia (Gulya and Viranyi, 1991). Race 5 was confined to green house (Ljubich, 1989). Race 7 was reported in Argentina (Gulya et al., 1991). Race 8 was reported in North Dakota (Miller and Gulya, 1991), that confers resistance to SDM races have been identified in the wild *Helianthus* species. In 1992 in this study a 12th resistant gene *PI12* was reported in the wild sunflower germplasm lines that confers resistance to SDM race 1. The objective of this project was to find out resistance of the inbred lines to SDM race 1, number of genes conferring resistance and their allelic relationship in the various inbred lines. *P. halstedii* completes a sexual cycle every season, where new pathogenic races may arise by mutation and recombination. Thus selection and breeding for resistance is a major objective of this study and is also the most economical and effective way to control this disease.

Materials and Methods

Four released USDA lines (DM-2, RHA 274, RHA 266, and HA 821), and three Plant Introductions (AMES 3235, PI 497950, PI 497938) were used for this study. The SDM reactions of the inbred lines are given in Table 1.

Table 1: Resistance reaction of inbred lines to sunflower downy mildew race 1

Inbred line	Race 1		
	No. of seedlings		
	<R	S	React
AMES 3235	54	0	R
PI 497250	42	0	R
PI 497938	39	0	R
DM-2	39	1	R
RHA 274	41	0	R
RHA 266	39	1	R
HA 821	0	40	S
Checks:			
IS 003	3	113	S
RHA	265	200	R

Table 2: Reaction to sunflower downy mildew race 1 of the 21 half-diallel F1 families of inbred lines

F ₁ cross	No. of plants		
	< R	S	React
DM-2/AMES 3235	20	0	R
DM-2/PI 497250	20	0	R
DM-2/PI 497938	19	1	R
HA 821/AMES 3235	20	0	R
HA 821/PI 497250	22	0	R
HA 821/PI 497938	20	0	R
HA 821/DM-2	20	0	R
HA 821/RHA 274	19	0	R
HA 821/RHA 266	18	0	R
PI 497250/AMES 3235	21	0	R
PI 497938/AMES 3235	21	0	R
PI 497938/PI 497250	20	0	R
RHA 274/AMES 3235	20	0	R
RHA 274/PI 497250	21	0	R
RHA 274/PI 497938	18	0	R
RHA 274/DM-2	20	1	R
RHA 266/AMES 3235	19	1	R
RHA 266/PI 497250	18	0	R
RHA 266/PI 497938	20	0	R
RHA 266/DM-2	20	0	R
RHA 266/RHA 274	20	0	R
Checks			
IS 003	0	80	S
RHA 265	20	0	R

< R = Resistant, S = Susceptible, React = Reaction

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Table 3: Segregation ratios and chi-square (χ^2) values of the F_2 families of resistant inbred lines inoculated with downy mildew race 1

Cross	Family No	No. of plants		χ^2 values		Probability
		<R	/S	Ratio	Value	
PI 497250/AMES 3235	1	232	0	1:0	0.000	> 0.99
	2	258	0	1:0	0.000	> 0.99
Pooled χ^2		490	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
PI 497938/AMES 3235	1	226	0	1:0	0.000	> 0.99
	2	137	1	1:0	0.000	0.95-0.98
Pooled χ^2		363	1	1:0	0.000	> 0.99
Heterogeneity χ^2					0.002	0.95-0.98
PI 497938/PI 497250	1	60	0	1:0	0.000	0.90-0.95
	2	60	0	1:0	0.000	> 0.99
Pooled χ^2		120	1	1:0	0.000	0.95-0.98
Heterogeneity χ^2					0.002	0.95-0.98
DM-2/AMES 3235	1	200	2	1:0	0.000	> 0.99
	2	278	2	1:0	0.008	0.90-0.95
Pooled χ^2		498	2	1:0	0.000	0.90-0.95
Heterogeneity χ^2					0.004	0.90-0.95
DM-2/PI 497250	1	120	0	1:0	0.000	> 0.99
	2	119	0	1:0	0.000	> 0.99
Pooled χ^2		239	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
DM-2/PI 497938	1	60	0	1:0	0.000	> 0.99
	2	60	0	1:0	0.000	> 0.99
Pooled χ^2		120	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
RHA 274/AMES 3235	1	60	0	1:0	0.000	> 0.99
	2	60	0	1:0	0.000	> 0.99
Pooled χ^2		120	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
RHA 274/PI 497250	1	208	0	1:0	0.000	> 0.99
	2	280	0	1:0	0.000	> 0.99
Pooled χ^2		488	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
RHA 274/PI 497938	1	234	1	1:0	0.001	0.95-0.98
	2	113	0	1:0	0.000	> 0.99
Pooled χ^2		347	1	1:0	0.000	> 0.99
Heterogeneity χ^2					0.001	0.95-0.98
RHA 274/DM-2	1	59	1	1:0	0.004	0.90-0.95
	2	60	0	1:0	0.000	> 0.99
Pooled χ^2		119	1	1:0	0.002	0.95-0.98
Heterogeneity χ^2					0.002	0.95-0.98
RHA 266/AMES 3235	1	60	0	1:0	0.000	> 0.99
	2	60	0	1:0	0.000	> 0.99
Pooled χ^2		120	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
RHA 266/PI 497250	1	219	1	1:0	0.000	> 0.99
	2	220	0	1:0	0.000	> 0.99
Pooled χ^2		439	1	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
RHA 266/RHA 274	1	60	0	1:0	0.000	> 0.99
	2	60	0	1:0	0.000	> 0.99
Pooled χ^2		120	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
RHA 266/PI 497938	1	55	5	15:1	0.160	0.50-0.70
	2	54	6	15:1	0.871	0.30-0.50
Pooled χ^2		109	11	15:1	1.280	0.20-0.30
Heterogeneity χ^2					0.249	0.50-0.70
HA 266/DM-2	1	56	4	15:1	0.018	0.80-0.90
	2	55	5	15:1	0.160	0.50-0.70
Pooled χ^2		111	9	15:1	0.142	0.70-0.80
Heterogeneity χ^2					0.036	0.80-0.90
Checks:	<R	/S	/React			
IS 003	13	647	S			
RHA 265	120	0	R			
<R = Resistant, /S = Susceptible, /React = Reaction						

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Table 4: Segregation ratios and chi-square (χ^2) values of the testcross families of resistant inbred lines inoculated with downy mildew race 1

Cross	Family No	No. of plants		χ^2 values		Probability
		<R	\mathcal{S}	Ratio	Value	
CmHA 821//PI 497250/	1	136	0	1:0	0.000	> 0.99
AMES 3235	2	180	0	1:0	0.000	> 0.99
Pooled χ^2		316	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
CmsHA 821//PI 497938/	1	180	0	1:0	0.000	> 0.99
AMES 3235	2	180	0	1:0	0.000	> 0.99
Pooled χ^2		360	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
CmsHA 821//PI 497938/	1	20	0	1:0	0.000	> 0.99
PI 497250	2	20	0	1:0	0.000	> 0.99
Pooled χ^2		40	0	1:0	0.000	> 0.99
Heterogeneity χ^2				1:0	0.000	> 0.99
Cms HA 821//DM-2/	1	179	1	1:0	0.001	0.95-0.98
AMES 3235	2	220	0	1:0	0.000	> 0.99
Pooled χ^2		399	1	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	0.95-0.98
CmsHA 821//	1	20	0	1:0	0.000	> 0.99
DM-2/PI 497250	2	20	0	1:0	0.000	> 0.99
Pooled χ^2		40	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
CmsHA 821//	1	20	0	1:0	0.000	> 0.99
DM-2/PI 497938	2	20	0	1:0	0.000	> 0.99
Pooled χ^2		40	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
CmsHA 821//	1	20	0	1:0	0.000	> 0.99
RHA 274/AMES3235	2	20	0	1:0	0.000	> 0.99
Pooled χ^2		40	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
CmsHA 821//	1	180	0	1:0	0.000	> 0.99
RHA 274/PI 497250	2	180	0	1:0	0.000	> 0.99
Pooled χ^2		360	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
CmsHA 821//	1	80	0	1:0	0.000	> 0.99
RHA 274/PI 497938	2	200	0	1:0	0.000	> 0.99
Pooled χ^2		280	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
CmsHA 821//	1	20	1	1:0	0.000	> 0.99
RHA 274/DM-2	2	20	0	1:0	0.000	> 0.99
Pooled χ^2		40	1	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
CmsHA 821//	1	20	0	1:0	0.000	> 0.99
RHA 266/AMES3235	2	20	0	1:0	0.000	> 0.99
Pooled χ^2		40	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
CmsHA 821//	1	160	0	1:0	0.000	> 0.99
RHA 266/PI 497250	2	100	0	1:0	0.000	> 0.99
Pooled χ^2		260	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
CmsHA 821//RHA 266	1	20	0	1:0	0.000	> 0.99
RHA 274/	2	20	0	1:0	0.000	> 0.99
Pooled χ^2		40	0	1:0	0.000	> 0.99
Heterogeneity χ^2					0.000	> 0.99
CmsHA 821//RHA 266	1	14	6	3:1	0.067	0.70-0.80
RHA 274/PI 497938	2	15	7	3:1	0.242	0.50-0.70
Pooled χ^2		29	13	3:1	0.508	0.30-0.50
Heterogeneity χ^2					0.199	0.50-0.70
CmsHA 821//RHA 266/	1	16	4	3:1	0.067	0.70-0.80
DM-2	2	15	5	3:1	0.000	> 0.99
Pooled χ^2		31	9	3:1	0.033	0.80-0.90
Heterogeneity χ^2					0.034	0.80-0.90
Checks:	<R	\mathcal{S}	\neq React			
IS 003	6	594	S			
RHA 265	120	0	R			

<R = Resistant, \mathcal{S} = Susceptible, \neq React = Reaction

Development of F₁, F₂ and Test Crosses: All the inbred lines were crossed in a half-diallel passion. A total of 21 F₁ crosses were produced in 1990, for the seven inbred lines. Two heads were used for each cross to avoid possible loss of a cross or shortage of seed for screening F₁ families against SDM race 1. Seeds from 21 F₁ hybrids were sown in the summer of 1991, to produce F₂ and testcrosses. A block of cmsHA821 also was planted to produce testcrosses. Six F₁ plant from each F₁ cross were visually identified and bagged before flowering to produce F₂ seed. Pollen from the six F₁ plants from a cross of resistant parents was crossed to cmsHA 821 to produce testcross heads. Hence, the same plants were used to develop F₂ and testcross heads. A total of 126 heads each for F₂ and testcross were completed in the field. At maturity each F₂ and testcross heads were harvested individually, dried in the drying room, threshed and cleaned separately, and stored in the cold room for use in spring 1992.

Seed Inoculation Technique: The whole seedling inoculation (WSI) technique, described by Gulya *et al.*, (1991) was used to provide a uniform infection load under controlled conditions. Seedlings with a radicle length of 10 to 15 mm and visible root hairs were inoculated by immersion for 3 hours at 18°C in a suspension containing 3 to 4 × 10⁴ zoospores/ml. Inoculated seedlings were grown in a mixture of sand and perlite (3:2 v/v) in a greenhouse (24 ± 3°C, 16 hours photoperiod) for 10 to 14 days. The seedlings were put overnight in the cold room maintained at 100% relative humidity and 18°C to effect sporulation.

Seedlings were evaluated as resistant or susceptible on the basis of the absence or presence of a visible white covering of sporangiophores and zoospores on cotyledons or true leaves. Results were considered more reliable when the susceptible check was 100% sporulated and resistant checks were 100% clear from sporulation.

Screening of inbred lines: Thirty nine to 54 seedlings from each of the inbred line were inoculated separately with SDM race 1 including a common susceptible check IS 003, and resistant check RHA 265. The seedlings from inbred lines were evaluated for their resistant and susceptible reactions to SDM race 1.

Screening of F₂ and Testcross Families: Sixty one to 280 F₂ and 20 to 220 seedlings of testcross (depending upon the seed availability) for each of the two families of a respective cross were inoculated separately with SDM race 1 including the same susceptible and resistant checks used for F₁s. If inheritance of resistance is controlled by two dominant genes, occurring at different loci and if each parent in a cross is homozygous for one allele at a time, we could expect 3 susceptible out of 60 seedlings in each F₂ family and 5 to 6 susceptible in 120 seedlings of two pooled families at 99% probability. No segregation would be expected if parents are homozygous. Results were analyzed according to χ^2 tests at the 99% level of probability, using the Yates correction factor for continuity described by Steel and Torrie (1981).

An insignificant number of unexpected susceptible plants were observed while testing the inbred lines and resistant families coming from F₂ and testcrosses. Zimmer (1974) reported upto 2% susceptible plants while screening resistant inbred lines against SDM in greenhouse.

Results and Discussion

Reaction of Inbred Lines to Sunflower Downy Mildew Race 1: Thirty-nine to 54 seedlings of the 7 inbred lines were

evaluated separately for resistance to SDM race 1. All the inbred lines were almost 100% resistant except HA 821 which was 100% susceptible (Table 1). Inbred lines IS 003 and RHA 265 were used as susceptible and resistant checks, respectively. One susceptible seedling each in DM-2 and RHA 266 could be explained by high inoculum concentration or due to modifier gene action as explained by Tan (1999).

Evaluation of F₁ Families From Crosses of Inbred Lines for Mode of Inheritance of Resistance to Sunflower Downy Mildew Race 1: Twenty one half diallel F₁ families of the 7 inbred lines were evaluated for resistance to SDM race 1 (Table 2).

All the F₁s were 100% resistant to SDM race 1 except DM-2/PI 497938, RHA 274/DM-2, and RHA 266/AMES 3235, each of which produced one unexpected susceptible plant. However, 95% or more of the plants of these crosses were resistant to SDM race 1. A resistant reaction of F₁s to SDM race 1 indicated that resistance to SDM 1 is due to dominant gene action and is simply inherited.

Allelic Relationships of Genes Conferring Resistance to Sunflower Downy Mildew Race 1 in Resistant Inbred Lines: To establish the allelic relationships of the SDM race 1 resistance genes in the resistant inbred lines, 61 to 280 seedlings of each of the two families of an F₂ cross and 20 to 220 seedlings of each of the two families of a testcross were evaluated for resistance to SDM race 1. Heterogeneity chi-square values were not significant (for families in the same cross), therefore families coming from the same cross were pooled for combined interpretation. Half diallel F₂ of PI 497250/AMES 3235, PI 497938/AMES 3235, PI 497938/PI 497250, DM-2/AMES 3235, DM-2/PI 497250, DM-2/PI 497938, RHA 274/AMES 3235, RHA 274/PI 497250, RHA 274/PI 497938, RHA 274/DM-2, RHA 266/AMES 3235, RHA 266/PI 497250, RHA 266/RHA 274 (Table 3) and their testcross families with cmsHA821 (Table 4) did not segregate. This suggested that AMES 3235, PI 497250, and RHA 274 have one gene in common with DM-2, PI 497938 and RHA 266.

However, each F₂ and testcross family of RHA 266/PI 497938 and RHA 266/DM-2 segregated in 15R:1S and 3R:1S ratios, respectively, for resistance to SDM race 1. This shows a two-gene segregation, each of these lines exhibited monogenic inheritance of resistance to SDM race 1. Therefore, PI₁ of RHA 266, reported by Gulya *et al.*, (1991), is non-allelic to the resistance gene in DM-2 and PI 497938. This also indicated that AMES 3235, PI 497250, and RHA 274, which did not segregate in F₂ and testcross with RHA 266, also have PI₁ of RHA 266. The other gene in these lines, which is the same as in DM-2 and PI 497938, also confers resistance to SDM race 1, which is designated as PI₁₂; a new gene.

Thus the two PIs, AMES 3235 and PI 497250, and a released line, RHA 274 have two genes (PI₁ and PI₁₂) that confer resistance to SDM race 1. DM-2 and PI 497938 each has PI₁₂ for resistance to SDM race 1 and RHA 266 has PI₁.

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