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Mode of Resistance and Number of Genes Conferring Resistance to Sunflower Downy Mildew Race 2 in Sunflower (*Helianthus annuus* L.)

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Abstract: Three USDA plant introductions; AMES 3235, PI 497250 and PI 497938, and four released lines, RHA 274, DM-2, RHA 266, and HA 821, were studied for resistance reaction and number of genes conferring resistance to SDM race 2. Digenic inheritance of resistance was found in AMES 3235, PI 497250, and RHA 274, where each of these lines were found to have two different genes for conferring resistance to SDM race 2, while DM 2 and PI 497938 each has one gene to inherit resistance to race 2. RHA 266 and HA 821 have no resistance at all to race 2.

Key words: *Helianthus annuus* L., *Plasmopara halstedii* Race 2, gene number and their inheritance.

Introduction

Sunflower downy mildew (SDM), *Plasmopara halstedii* (Farl.) Berl and de Toni, originated in North America (Leppik, 1966) and spread throughout the growing regions worldwide. The disease reduces both yield and oil quality in USA, Canada, and Europe (Sackston *et al.*, 1990). Resistance to *P. halstedii* in cultivated sunflower has been found in wild *Hellianthus. annuus* L. and cultivated sunflower germplasm lines (Sackston, 1981 and Sackston *et al.*, 1990). Resistance to SDM was controlled by a single dominant gene in an interspecific hybrid between *Helianthus annuus* L. (2n = 34) and *H. tuberosus* L. (2n = 102) (Pustovoit and Kroknin, 1978). Race 2 of SDM, *Plasmopara halstedii* (Farl) Berl and de Toni was identified by Vear and Leclercq (1971), when they reported the presence of two independent dominant genes, H1 and H2, later on designated as PI_2 and PI_3 respectively, in inbred line HA *61*. These were different from the PI gene in AD *66*, which Zimmer and Kinman (1972) reported to be PI_1. All the three genes were effective against SDM in France (Vear and Leclercq, 1971). Resistance to the North American and European races of SDM is traced to wild *H. annuus* L. (Zimmer, 1974) and other wild species (Miller and Gulya, 1988). The resistance in some species may be due to the PI_2. Some of the resistant species were also collected in Texas, where the PI_2 resistant materials were also found (Zimmer and Kinman, 1972). Thompson *et al.* (1978) reported that *Helianthus praecox* ssp. *runyonii, H. praecox ssp. hirtus, H. argophyllus,* and 18 wild *H. annuus* L. entries were resistant to race 2 of downy mildew. Resistance to SDM race 2 was most common among 5 perennial species (*H. tuberosus, H. rigidus, H. grossesserratus, H. maximiliani, and H. nuttallii,* whereas the annual species were highly susceptible (Fick *et al.*, 1974).

The "group immunity" cultivars "Novinka" and "Progress" developed byPustovoit *et al.* (1976) are reported to have resistance to SDM, derived from *H. tuberosus* L. and resistance was controlled by a single gene. Later Miller and Gulya (1987) reported that these two cultivars had PI_5, which gives resistance to races 2 and 3 of SDM. Zimmer and Kinman (1972) reported successful transfer of resistance from *H. tuberosus* to *H. annuus* and released HIR *34* with PI_4 as a SDM race 2 resistant line, with a chromosome number of 2n = 24. Vear and Leclercq (1971) also developed SDM resistant source by crossing *H. tuberosus* with *H. annuus* species. The objectives of experiment were to determine the inheritance and number of the genes conferring resistance to SDM race 2 in the inbred lines.

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) obtained from the Plant Introduction Station, Iowa State University, Ames Iowa, were used for study. Lines were planted in the field at North Dakota State University Research Farm Fargo, in the Summer 1990 were intercrossed to produce F1 seeds.

Crossing techniques: All inbred lines were fertile. Heads from every female parent for each F_1 cross were covered with cloth bags before flowering. Flowering heads were emasculated daily by removing anther tubes and were washed with water to remove remnant pollens to avoid self-pollination. Heads were kept covered with bags after emasculation to avoid out crossing until all the florets on the heads had been completely emasculated. The emasculated heads were pollinated two days after the first day of emasculation and then each day with fresh pollen of the male parent. Crossed heads were labeled and kept covered with bags to avoid out crossing and damage by birds until maturity.

Development of F_1, F_2 and BC_1F_1 crosses: All the inbred lines were crossed in a half-diallel passion. A total of 20 F_1 (at least one parent in a cross was resistant to SDM race 2) crosses were produced in summer 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 F_1 families against SDM race 2. The seed harvested was put in separate envelops and kept in the cold room at 7 to 8°C until used the next summer, 1991. Seeds from 20 F_1 hybrids were sown in the summer of 1991, to produce F_2 and BC_1F_1 crosses. A block of Cms HA *821* also was planted to produce F_2 and BC F_1 crosses. Six F_1 plants from each F_1 cross were visually identified and bagged before flowering to produce F_2 seeds. Pollens of the six F_1 plants from a cross of resistant parents were crossed to Cms HA *821* to produce BC_1F_1 heads. A total of 60 heads for F_2 and test crosses were completed in the field. At maturity each F_2 and BC_1F_1 head was harvested individually, dried, threshed and cleaned separately, and stored in the cold room.

Seed germination: Seeds were surface sterilized in about 1% sodium hypochlorite solution for 10 min. and soaked in 0.8 ml "Etherel" (2-Chloroethyl phosphonic acid) per liter of water for 18 to 24 hours and washed with cold water for 2 to 3 minutes to remove Etherel residue, separately placed on moist seed germination blotting papers, rolled to form "ragdolls," and were put in the germinator at 27 to 28°C for germination. The following day germinated seedlings (10 to 15 mm long root with visible root hairs) were picked and placed in separate labeled petri dishes. A small amount of distilled water was added to the seedlings in the petri dishes to keep them moist, and the dishes were placed in refrigerator (5°C) to stop the

seedling growth. The remaining seeds on the blotting papers were moistened again, wrapped, and returned to the germinator. By the second day, seedlings of the proper size were selected and mixed with their respective seeds in the petri dishes in the refrigerator.

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 h at 18°C in a suspension containing 3 to 4 x 10^4 zoosporangia/ml. Inoculated seedlings were grown in a mixture of sand and perlitre (3:2 v/v) in a greenhouse (24 ± 3°C, 16 h 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 the sporulation. Seedlings were evaluated as resistant or susceptible on the basis of the absence or presence of a visible white covering of sporangiophores and zoosporangia on cotyledons or true leaves.

Genetics of resistance to sunflower downy mildew race 2 screening of inbred lines and F1 families: Forty seedling from each of the inbred lines and F1 families were inoculated separately with SDM race 2, including a common susceptible check, IS 003 and resistant check RHA 274, and also DM2 in case of F1 families were evaluated for susceptible and resistant reaction against SDM race 2.

Screening of F_2 and BC_1F_1 families: 60 to 250 F_2 and 20-140 BC_1F_1 seedlings for each of the two families of a cross were inoculated separately with SDM race 2 including the same susceptible and resistant checks used for inbred lines. 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 susceptibles out of 60 seedlings in each F_2 family and 5 to 6 susceptibles in 120 seedlings of two pooled families at 99% probability. No segregation would be expected if parents are homozygous. After screening, some F_2 crosses were found to segregate in ratios suggesting 2 or more genes. Therefore, testing for these crosses was repeated with an increased number of seedlings (according to the availability of the seeds) for proper statistical analysis and meaningful interpretation. Results were analyzed according to x^2 tests at the 99% level of probability, using the Yates correction factor for continuity (Steel and Torrie, 1981).

Results and Discussion

Reaction of inbred lines to SDM race 2: Forty seedlings each of the inbred lines were studied for resistance to SDM race 2 (Table 1). AMES 3235, PI 497250, PI 497938, DM-2, and RH 274, which were found 100% resistant and RHA 266 and HA 821 were 100% susceptible to SDM race 2. The susceptible check (IS 003) showed more than 97% susceptible reaction and resistant check (RHA 274) gave 100% resistant reaction.

Evaluation of F_1 families from crosses of inbred lines for mode of inheritance of resistance to SDM race 2: Twenty one half diallel F_1 families were evaluated for resistance to SDM race 1 (Table 2).

All the F_1s were 100% resistant to SDM race 2 except RHA 274/DM-2, and RHA 266/PI 497250, each of which produced one unexpected susceptible plant. However, 95% or more of the plants of these crosses were resistant to SDM race 2.

A resistant reaction of F_1s to SDM race 2 indicated that resistance to SDM 2 is due to dominant gene action and is simply inherited.

Table 1: Resistance reaction of sunflower inbred lines to SMD race 2.

Inbred Line	Race 2 (No. of seedlings)		
	<R	AS	TReact
AMES 3235	40	0	R
PI 497250	40	0	R
PI 497938	40	0	R
DM-2	40	0	R
RHA 274	40	0	R
RHA 266	0	40	S
HA 821	0	40	S
Check			
IS 003	3	113	S
RHA 274	40	0	R

<R = Resistant, AS = Susceptible, TReact = Reaction

Table 2: Reaction to sunflower downy mildew race 2 of the 20 half-diallel F_1 families of inbred lines

F1 cross	No. of plants		
	<R	AS	TReact
DM-2/AMES 3235	20	0	R
DM-2/PI 497950	20	0	R
DM-2/PI 497938	20	0	R
HA 821/AMES 3235	20	0	R
HA 821/PI 497250	20	0	R
HA 821/PI 497938	20	0	R
HA 821/DM-2	20	0	R
HA 821/RAH 274	20	0	R
PI 497250/AMES 3235	19	0	R
PI 497938/AMES 3235	20	0	R
PI 497938/PI 497250	20	0	R
RAH 274/AMES 3235	20	0	R
RAH 274/PI 497250	20	0	R
RAH 274/PI 497938	20	0	R
RAH 274/DM-2	20	1	R
RAH 266/AMES 3235	20	0	R
RAH 266/PI 497250	19	1	R
RAH 266/PI 497938	20	0	R
RAH 266/DM-2	20	0	R
RAH 266/RAH 274	20	0	R
Check			
IS 003	2	78	S
DM-2	20	0	R
RHA 274	20	0	R

<R = Resistant, AS = Susceptible, TReact = Reaction

Number of genes, conferring resistance to SDM race 2 in resistant inbred lines: Heterogeneity chi-square values (for the families coming from the same cross) were not significant. Therefore, data for different families in the same cross were pooled for combined analysis.

F_2 families of HA 821/AMES 3235, HA 821/PI 497250, and HA 821/RHA 274 segregated in 15R : 1S in F2 and 3R : 1S in BC_1F_1 suggesting that resistance to SDM race 2 in lines AMES 3235, PI 497250, and RHA 274 are controlled by two independent dominant genes. Whereas, families of HA 821/PI 497938 and HA 821/DM-2 segregated in 3R:1S ratios in the F_2 and 1:1S in the BC_1F_1, indicating that PI 497938 and DM-2 each has a single dominant gene for resistance to SDM race 2 (Table 3 and 4).

It was concluded that each of the inbred lines AMES 3235, PI

Table 3: Segregation ratios and chi-square (χ^2) values of the F2 families derived from crosses of SDM race 2 resistant inbred lines with the susceptible line HA 821 after inoculation with SDM race 2.

Cross		No. of plants		χ^2 values		
	Family No	<R	AS	Ratio	Value	Probability
HA 821/AMES 3235	1	121	5	15:1	0.764	0.30-0.50
	2	88	2	15:1	1.852	0.10-0.20
Pooled χ^2		209	7	15:1	2.844	0.05-0.10
Heterogeneity χ^2					0.228	0.50-0.70
HA 821/PI 497250	1	194	9	15:1	0.854	0.30-0.50
	2	117	3	15:1	2.276	0.10-0.20
Pooled χ^2		311	12	15:1	3.123	0.50-0.10
Heterogeneity χ^2					0.007	0.90-0.95
HA 821/RHA 274	1	185	10	15:1	0.250	0.50-0.70
	2	239	11	15:1	1.162	0.20-0.30
Pooled χ^2		424	21	15:1	1.527	0.20-0.30
Heterogeneity χ^2					0.115	0.70-0.80
HA 821/PI 497938	1	44	16	3:1	0.022	0.80-0.90
	2	48	12	3:1	0.555	0.30-0.50
Polled χ^2		92	28	3:1	1.100	0.70-0.80
Heterogeneity χ^2					0.477	0.30-0.50
HA 821/DM-2	1	46	14	3:1	0.022	0.80-0.90
	2	44	16	3:1	0.022	0.80-0.90
Pooled χ^2		90	30	3:1	0.000	>0.99
Heterogeneity χ^2					0.044	0.80-0.90
Checks		<R	AS	тReact		
IS 003		5	274	S		
RHA 274		110	0	R		

<R = Resistant, AS = Susceptible, тReact = Reaction

Table 4: Segregation ratios and chi-square (χ^2) values of the BC_1F_1 families of crosses of downy mildew race 2 resistant inbred lines with cmsHA 821 as the recurrent parent after inoculation with SMD race 2

Cross		No. of plants		χ^2 values		
	Family No	<R	AS	Ratio	Value	Probability
cmsHA 821/HA 821 /	1	101	26	3:1	1.57	0.20-0.30
AMES 3235	2	85	23	3:1	0.605	0.30-0.50
Pooled χ^2		186	49	3:1	1.841	0.10-0.20
Heterogeneity χ^2					0.179	0.50-0.70
cmsHA 821/HA 821 /	1	93	24	3:1	1.028	0.30-0.50
PI 497250	2	113	27	3:1	2.143	0.10-0.20
Pooled χ^2		206	51	3:1	3.373	0.05-0.10
Heterogeneity χ^2					0.202	0.50-0.70
cmsHA 821/HA 821 /	1	108	28	3:1	0.187	0.20-0.30
RHA 274	2	35	8	3:1	0.628	0.30-0.50
Pooled χ^2		143	36	3:1	2.028	0.10-0.20
Heterogeneity χ^2					0.213	0.50-0.00
cmsHA 821/HA 821 /	1	10	10	1:1	0.000	>0.99
PI 497938	2	12	8	1:1	1.450	0.50-0.70
Pooled χ^2		22	18	1:1	2.225	0.50-0.70
Heterogeneity χ^2					0.225	0.50-0.70
cmsHA 821/HA 821 /	1	10	10	1:1	0.000	>0.99
DM-2	2	11	9	1:1	0.050	0.80-0.90
Pooled χ^2		21	19	1:1	0.025	0.80-0.90
Heterogeneity χ^2					0.025	0.80-0.90
Checks		<R	As	т React		
IS 003		0	240	S		
RHA 274		100	0	R		

<R = Resistant, AS = Susceptible, тReact = Reaction

497250 and RHA 274 has two independent genes for resistance to SDM race 2. Whereas, lines DM 2 and PI 497938 each has one dominant gene for resistance to SDM race 2.

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