Gene *Pu*$_6$: A New Gene in Sunflower for Resistance to *Puccinia helianthi*

S. M. Yang, W. M. Dowler, and A. Luciano

First and second authors: research plant pathologist and supervisory plant pathologist, respectively, U.S. Department of Agriculture, Agricultural Research Service, Foreign Disease-Weed Science Research, Ft. Detrick, Frederick, MD 21701; third author: senior breeder, Argentina Criadero de Semillas, S.A., Parana, Entre Rios, Argentina.

The authors wish to express their appreciation to Drs. L. E. Browder, G. E. Holcomb, Rama R. Urs, and W. E. Sackston for their suggestions during the preparation of the manuscript.

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Accepted for publication 21 November 1988 (submitted for electronic processing).

ABSTRACT

Yang, S. M., Dowler, W. M., and Luciano, A. 1989. Gene *Pu*$_6$: A new gene in sunflower for resistance to *Puccinia helianthi*. Phytopathology 79:474-477.

Studies were conducted to determine the genetic basis of resistance of sunflower (*Helianthus annuus*) inbred line P386 to *Puccinia helianthi*. This study showed that P386 carries a single dominant gene conditioning resistance to the four North American (NA) races of *P. helianthi*. We propose the gene symbol *Pu*$_n$, instead of R_n, to designate the sunflower genes for resistance to *P. helianthi*, and the name *Pu*$_6$ for the resistance gene in P386. This gene in P386 was different from the resistance gene R_1 (*Pu*$_1$) in CM 90RR, conferring resistance to NA races 1 and 2, and gene R_2 (*Pu*$_2$) in CM 29-3, conferring resistance to NA races 1 and 3. The resistance gene in P386 was also different from gene R_4 (*Pu*$_4$) in the sunflower inbred line HAR$_3$ and gene R_5 (*Pu*$_5$) in HAR$_2$.

Additional keywords: inheritance, rust.

Sunflower (*Helianthus annuus* L.) lines can react differentially to North American (NA) races of *Puccinia helianthi* Schw., the causal organism of sunflower rust. CM 90RR, containing the gene R_1, is resistant to NA races 1 and 2 of *P. helianthi*, and CM 29-3, with R_2, is resistant to NA races 1 and 3 (Table 1) (5). The inbred line 403-4, with gene R_3, has been shown resistant to *P. helianthi* (1,4), but the R_3 resistance cannot be evaluated further because of the loss of this line (J. F. Miller, *personal communication*). Gene R_4, in the sunflower inbred lines HAR$_1$, HAR$_3$, HAR$_4$, and HAR$_5$, and gene R_5, in HAR$_2$, condition resistance to NA race 4 (4,6). Recently Yang (8) and Yang et al (9) found that the sunflower inbred line P386 was resistant to NA races 1–4 (Table 1). The objective of this research was to determine the resistance gene in P386 conferring resistance to the four NA races of *P. helianthi*.

MATERIALS AND METHODS

The resistant sunflower inbred line P386 was crossed with the susceptible sunflower inbred line 89A; with the sunflower rust differential CM 90RR, having gene R_1; and with the rust differential CM 29-3 or the cultivar Morden 307-1, having gene R_2. Seedlings of these crosses were grown in 10- or 15-cm clay pots, unless otherwise stated, in the greenhouse (22 ± 2 C). Seedlings of CM 29-3 (or Morden 307-1), CM 90RR, P386, and S37-388 (a universal suscept) grown in 10-cm pots were included in each test in order to check the purity of each isolate of *P. helianthi*: S37-388 is susceptible to NA race 1, both S37-388 and CM 29-3 (or Morden 307-1) are susceptible to NA race 2, both S37-388 and CM 90RR

TABLE 1. Reactions of four sunflower lines to four North American races of *Puccinia helianthi* (5,9)

	Race[a]			
Sunflower line	1	2	3	4
P386	R	R	R	R
CM 90RR	R	R	S	S
CM 29-3	R	S	R	S
S37-388	S	S	S	S

[a] R indicates resistant reactions: no infection, no pustules (type 0 reaction), pustules less than 0.2 mm in the broadest part (type 1 reaction), or pustules 0.2–0.4 mm (type 2 reaction). S indicates susceptible reactions: pustules 0.4–0.6 mm (type 3 reaction) or pustules larger than 0.6 mm (type 4 reaction).

are susceptible to NA race 3, and all four inbred lines are susceptible to NA race 4 (5).

Reaction tests were made, unless otherwise stated, on parental stocks, F_1, F_2, backcross F_1, and backcross F_2, with the seedlings (five to 30 per pot) at stage VE (the first leaf beyond the cotyledons but less than 4 cm long) to V2 (the first pair of true leaves at least 4 cm long, and the second pair less than 4 cm long) (7). The seedlings were separately atomized with aqueous urediniospore suspensions (10^4 to 10^5 urediniospores per milliliter) of NA races 1–4 of *P. helianthi*. The number of seedlings used in each test depended on the availability of seeds of each specific cross and the availability of progeny. Each cross and progeny tests were repeated at least twice.

Inoculated seedlings were incubated 18 to 22 hr at 100% relative humidity in darkness in 61-× 51-× 46-cm metal boxes in a growth chamber. During this period each box contained water about 2 to 3 cm deep and was covered with a metal lid. The boxes were then uncovered, water drained from them, and the plants maintained in the same boxes (one box for each race, containing nine 15-cm pots and three 10-cm pots) for an additional 13 days in the same growth chamber (12-hr light, 65 to 100% relative humidity, 21 ± 1 C day, 17 ± 1 C night).

A transparency chart showing black dots 0.2, 0.4, and 0.6 mm in diameter (8) was used to characterize the pustules produced on the inoculated leaves. Inoculated seedlings that showed no sign of infection (type 0 reaction) or produced pustules less than 0.2 mm in diameter (type 1 reaction) or pustules 0.2–0.4 mm in diameter (type 2 reaction) were classified as resistant. Those that produced pustules 0.4–0.6 mm in diameter (type 3 reaction) or pustules larger than 0.6 mm in diameter (type 4 reaction) were classified as susceptible (5,8) (Table 1).

S37-388, CM 29-3, and CM 90RR were obtained from the Seed Stocks Project, North Dakota Agricultural Experiment Station, North Dakota State University, Fargo, in 1985 and again in November 1987. The four NA races of *P. helianthi* were obtained from T. Gulya, USDA-ARS, North Dakota State University, in 1986 and 1987.

In order to determine whether the resistance gene in P386 is similar to the gene R_4 in HAR_3, seeds of F_1 and F_2 progeny from two crosses between P386 × HAR_3 and HAR_3 were planted in soil in a 10-cm-diameter pot. Seedlings (one or two per pot) at stages V2 and V3 (7) were inoculated with urediniospores of NA races 1–4 of *P. helianthi*. The cotton plug method (3) was used to inoculate the seedlings. A small plug of sterile, wet absorbent cotton touched to freshly collected urediniospores in a petri dish was lightly pressed into the adaxial surface of the second or third pair of true leaves. Each leaf was inoculated with two races: one on one side of the midvein and one on the other side. Each seedling was inoculated with the four cultures of *P. helianthi*. After inoculation, the seedlings, in pots, were placed in a dew chamber in darkness at 20 C for 16 to 20 hr. Then the seedlings were removed from the dew chamber and maintained on greenhouse benches (19 ± 2 C) for an additional 13 days.

A single-dominant-gene inheritance for resistance in P386 was tested by using chi-square tests of goodness of fit and heterogeneity.

RESULTS AND DISCUSSION

All seedlings of P386 and of F_1 of 89A × P386 were resistant

TABLE 2. Seedling reaction of F_2 populations of 89A × P386 (susceptible and resistant parents, respectively) to four North American races of *Puccinia helianthi*

	Race[a]											
	1			2			3			4		
Cross	R[b]	S[b]	*P* value[c]	R	S	*P* value	R	S	*P* value	R	S	*P* value
1	37	15	0.50–0.70	103	38	0.50–0.70	108	42	0.30–0.50	111	43	0.30–0.50
2	30	12	0.50–0.70	87	25	0.50–0.70	127	46	0.50–0.70	120	45	0.50–0.70
3	43	10	0.30–0.50	126	48	0.30–0.50	118	43	0.50–0.70	149	55	0.50–0.70
4	—[d]	—		78	28	0.70–0.80	83	29	0.80–0.90	76	33	0.20–0.30
5	—	—		82	27	0.95–0.98	89	38	0.20–0.30	70	26	0.50–0.70
6	—	—		89	26	0.50–0.70	102	37	0.50–0.70	85	24	0.30–0.50
Heterogeneity chi-square			0.30–0.50			0.80–0.90			0.95–0.98			0.80–0.90

[a] Seedling reaction to North American race 1 was repeated only once, but reactions to others were repeated at least twice.
[b] R indicates resistant reactions (reaction types 0 to 2); S indicates susceptible reactions (reaction types 3 and 4).
[c] Based on an expected 3:1 ratio of resistant to susceptible plants.
[d] No test was made.

TABLE 3. Seedling reaction of test cross F_1 and segregating F_2 progenies of 89A × (89A × P386) to three North American races of *Puccinia helianthi*

		Race								
		2			3			4		
	Test cross	R[a]	S[a]	*P* value[b]	R	S	*P* value	R	S	*P* value
F_1	1	54	63	0.30–0.50	67	59	0.30–0.50	67	57	0.30–0.50
	2	68	60	0.30–0.50	79	70	0.30–0.50	74	68	0.50–0.70
	3	62	68	0.50–0.70	73	64	0.30–0.50	69	77	0.50–0.70
	4	67	75	0.50–0.70	69	75	0.50–0.70	78	72	0.50–0.70
Heterogeneity chi-square				0.50–0.70			0.70–0.80			0.70–0.80
Segregating F_2	1	56	21	0.50–0.70	73	21	0.50–0.70	85	24	0.30–0.50
	2	79	23	0.50–0.70	80	30	0.50–0.70	82	29	0.70–0.80
	3	68	20	0.50–0.70	79	23	0.50–0.70	75	29	0.30–0.50
	4	72	29	0.30–0.50	76	29	0.50–0.70	80	25	0.70–0.80
Heterogeneity chi-square				0.50–0.70			0.70–0.80			0.70–0.80

[a] R indicates resistant reactions (reaction types 0 to 2); S indicates susceptible reactions (reaction types 3 and 4).
[b] Based on an expected 1:1 ratio of resistant to susceptible plants in F_1 progeny and 3:1 ratio of resistant to susceptible plants in segregating F_2 progeny.

(reaction type 0, 1, or 2) to NA races 1–4 of *P. helianthi*. Both resistant and susceptible reactions (reaction type 3 or 4) to the four races were observed in F_2 populations. The distribution of resistant to susceptible plants in F_2 progeny showed good fit to a 3:1 ratio (Table 2), indicating that P386 has a single dominant gene conferring resistance to each race. A heterogeneity chi-square test of F_2 population data from three inoculations with NA race 1 produced a *P* value of 0.30–0.50; with NA races 2–4, a *P* value of 0.80–0.90 or more.

In F_1 test crosses of 89A × (89A × P386), the distribution of resistant to susceptible plants to NA races 2–4 showed good fit to 1:1 (Table 3). The segregating test cross F_2 population obtained from the four F_1 test crosses yielded segregation ratios that fitted a 3:1 ratio of resistant to susceptible plants (Table 3). The heterogeneity chi-square test also indicated that the four F_1 test crosses and the segregating F_2 crosses came from the same population. This result confirms the presence of a single dominant gene in P386 for resistance to sunflower rust. The reaction of F_1 of 89A × (89A × P386) to NA race 1 was not determined because of a lack of seeds.

All the F_1 plants of the cross between P386 and the differential CM 90RR (having gene R_1) were also resistant to NA races 1–4 of *P. helianthi*. The 3:1 segregations in F_2 populations of this cross with NA races 3 and 4 and the 15:1 segregation with NA races 1 and 2 (Table 4) indicate that P386 has a single dominant gene different from and independent of R_1. Similar results from the cross between P386 and CM 29-3 (having gene R_2) indicate that the gene in P386 is not R_2 (Table 5).

All seedlings of HAR_3 and F_1 of 89A × HAR_3 were resistant to the four NA races of *P. helianthi*. However, in F_2 populations of 89A × HAR_3 some seedlings were resistant and some were susceptible to the four races. The distribution of resistant to susceptible plants in F_2 progeny showed good fit to a 3:1 ratio (S. M. Yang, *unpublished data*). This result indicates that the single dominant gene R_4 confers resistance in HAR_3 to NA race 4 (4) and also confers resistance to the other three NA races. All seedlings of F_1 of P386 × HAR_3 were also resistant to the four NA races. However, both resistant and susceptible reactions to the four races were observed in F_2 populations: the 15:1 segregation in two crosses with NA race 2 and in one of the two crosses with NA races 1, 3, and 4 (Table 6). This result indicates that the resistant gene in P386 is different from the gene R_4, in HAR_3. The lack of segregation in one of the two crosses with NA races 1, 3, and 4 might be due to the insufficient number of available seeds used.

The aforementioned cotton plug method was also used to inoculate 50 seedlings each of the sunflower inbred lines HAR_1, HAR_2, HAR_3, HAR_4, HAR_5, S37-388, Morden 307-1, and CM 90RR with NA race 1. All HAR_2, HAR_5, and S37-388 seedlings were susceptible, but the others were resistant to NA race 1. Similar results were also obtained from 50 seedlings of each of the eight inbred lines inoculated with urediniospores of NA race 1 by means of the spore-settling tower. This result indicates that gene R_5, in HAR_2, is ineffective against NA race 1. P386 has one gene that confers resistance to NA race 1. Therefore, R_5 and the gene in P386 cannot be the same.

Putt and Sackston (5) first identified genes in sunflower inbred lines resistant to four NA cultures of *P. helianthi* and used R_1 to designate the resistance gene in CM 90RR that conditions resistance to NA races 1 and 3, and R_2 the gene in CM 29-3 that conditions resistance to NA races 1 and 2. The third gene, R_3, was found in the sunflower line 403-4 by other Canadian workers (1). R_4 and R_5 were found by North Dakota scientists (4,6), who also

TABLE 4. Seedling reaction of F_2 populations of crosses between the sunflower rust differential CM 90RR (having gene R_1) and the sunflower line P386 to four North American races of *Puccinia helianthi*

	Race											
	1			2			3			4		
Cross	R[a]	S[a]	*P* value[b]	R	S	*P* value[b]	R	S	*P* value[c]	R	S	*P* value[c]
1	47	4	0.50–0.70	48	2	0.50–0.70	42	17	0.30–0.50	41	11	0.50–0.70
2	44	2	0.50–0.70	39	4	0.30–0.50	45	12	0.30–0.50	37	10	0.50–0.70
3	54	4	0.80–0.90	50	2	0.30–0.50	29	8	0.50–0.70	39	14	0.80–0.90
4	—[d]	—	—	44	5	0.20–0.30	42	16	0.50–0.70	48	12	0.30–0.50
5	—	—	—	47	2	0.50–0.70	38	15	0.50–0.70	44	13	0.70–0.80
6	—	—	—	40	3	0.80–0.90	42	13	0.80–0.90	41	12	0.50–0.70
Heterogeneity chi-square			0.70–0.80			0.50–0.70			0.80–0.90			0.98–0.99

[a] R indicates resistant reactions (reaction types 0 to 2); S indicates susceptible reactions (reaction types 3 and 4).
[b] Based on an expected 15:1 ratio of resistant to susceptible plants.
[c] Based on an expected 3:1 ratio of resistant to susceptible plants.
[d] No test was made.

TABLE 5. Seedling reaction of F_2 populations of crosses between the sunflower rust differential CM 29-3 (having gene R_2) and the sunflower line P386 to four North American races of *Puccinia helianthi*

	Race											
	1			2			3			4		
Cross	R[a]	S[a]	*P* value[b]	R	S	*P* value[c]	R	S	*P* value[b]	R	S	*P* value[c]
1	35	2	0.80–0.90	35	10	0.50–0.70	41	2	0.50–0.70	38	13	0.90–0.95
2	40	2	0.70–0.80	33	12	0.70–0.80	38	2	0.70–0.80	44	19	0.30–0.50
3	27	3	0.30–0.50	23	6	0.50–0.70	46	4	0.50–0.70	22	9	0.50–0.70
4	—[d]	—	—	19	7	0.80–0.90	18	2	0.30–0.50	19	8	0.50–0.70
5	—	—	—	40	11	0.50–0.70	37	2	0.70–0.80	47	18	0.50–0.70
6	—	—	—	38	15	0.50–0.70	38	4	0.30–0.50	43	13	0.70–0.80
Heterogeneity chi-square			0.50–0.70			0.90–0.95			0.80–0.90			0.95–0.98

[a] R indicates resistant reactions (reaction types 0 to 2); S indicates susceptible reactions (reaction types 3 and 4).
[b] Based on an expected 15:1 ratio of resistant to susceptible plants.
[c] Based on an expected 3:1 ratio of resistant to susceptible plants.
[d] No test was made.

TABLE 6. Seedling reaction of F_2 populations of crosses between the sunflower lines P386 and HAR_3 to four North American races of *Puccinia helianthi*

	Race											
	1			2			3			4		
Cross	R[a]	S[a]	*P* value[b]	R	S	*P* value	R	S	*P* value	R	S	*P* value
1	43	0	0.05–0.20	39	4	0.30–0.50	43	0	0.05–0.20	43	0	0.05–0.20
2	66	3	0.50–0.70	63	6	0.30–0.50	66	3	0.50–0.70	67	2	0.20–0.30

[a] R indicates resistant reactions (reaction types 0 to 2); S indicates susceptible reactions (reaction types 3 and 4).
[b] Based on an expected 15:1 ratio of resistant to susceptible plants.

used *R* in sequence to designate sunflower inbred lines that are resistant to *P. helianthi* (2): HAR_1, HAR_3, HAR_4, and HAR_5, each having R_4, and HAR_2, having R_5.

As the number of sunflower genotypes increases, the continued use of *R* to designate the resistance genes to rust and to name rust-resistant inbred lines will cause confusion. Since we are studying the resistance in sunflower to *P. helianthi*, we propose using *Pu* in sequence (i.e., Pu_1, Pu_2, Pu_3, . . . , Pu_n, rather than R_1, R_2, R_3, . . . , R_n) to designate the sunflower genes for resistance to *P. helianthi*.

The resistance gene in P386 is different from the resistance genes Pu_1 (R_1), Pu_2 (R_2), Pu_4 (R_4), and Pu_5 (R_5). We therefore name the resistance gene in P386 Pu_6. Pu_6 confers a broader resistance to sunflower rust than any of the previously described genes. This is apparently related to a greater frequency in current *P. helianthi* populations of the genes for virulence corresponding to the previously described resistance genes.

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