Transferring Powdery Mildew Resistance Genes from Wild *Helianthus* into Cultivated Sunflower

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Introduction

Powdery mildew (PM) (*Erysiphe chicoracearum* D.C.), a widely distributed pathogen of cultivated sunflower, frequently causes economic loss in warmer climates (Zimmer and Hoes, 1978). The development of PM resistant cultivars is a promising solution that may allow production zones to be expanded. Sunflower cultivars differ in their reaction to PM, but resistant cultivars are not available and relatively little effort has been devoted to develop resistant germplasm. Perennial wild sunflower species, *Helianthus californicus*, *H. ciliaris*, *H. decapetalus*, *H. lacinatus*, *H. rigidus*, *H. grosseserratus*, *H. maximiliani*, and annual wild species, such as *Helianthus debilis* ssp. *silvestris*, *H. praecox* ssp. *praecox*, and *H. bolanderi* are valuable sources of PM resistance genes (Saliman et al., 1982; Christov et al., 1996). Jan and Chandler (1985) and Rojas-Barros et al. (2004) have been recognized two accessions of *H. debilis* ssp. *debilis* as sources of resistance to PM which can be transferred into cultivated sunflower. Jan and Chandler (1985) found the PM resistance to be incompletely dominant and incorporated it into a cultivated background which was released as the germplasm PM1 (Jan and Chandler, 1988).

This report summarizes efforts to identify and transfer the PM resistance observed in interspecific crosses between wild annual *Helianthus* species and cultivated sunflower, and to obtain preliminary information on the inheritance of PM resistance.

Materials and Methods

Three PM resistant plants from *H. debilis* ssp. *debilis*, accession PI 435671, and one PM resistant plant from *H. argophyllus*, accession PI 494582, were identified in the late fall of 2003. Single heads from these four plants were collected 5 days after pollination with bulk pollen from the susceptible line HA 89 to produce the F_1 generation using the embryo rescue technique (Jan, 1997). In the winter of 2003-04, 17 BC_1F_1 plants from the most PM resistant F_1 plants derived from one of the *H. debilis* × HA 89 crosses (Rojas-Barros et al., 2003) were self-pollinated to produce BC_1F_2 populations.

Helianthus argophyllus plants began flowering in December 2003, six months after *H. debilis*, and F_1 seed from the *H. argophyllus* × HA 89 cross were produced in January. In the spring of 2004, F_1 plants from the *H. argophyllus* × HA 89 cross were pollinated with HA 89 pollen to produce BC_1F_1 seeds.

In order to identify the segregating BC_1F_2 families and to conduct preliminary inheritance studies of the identified PM resistances, the following plant materials were evaluated in the greenhouse during the summer of 2004:

1. Parents
2. F_1 plants
3. 20 BC_1F_2 plants from each of the six *H. debilis* × HA 89 BC_1F_1 plants
4. 15 BC_1F_1 plants from each F_1 of *H. argophyllus* × HA 89 cross

Inoculation by rubbing the surface of the first fully-developed leaf with fresh spores was necessary due to a low incidence of natural infection during the summer of 2004. Additional inoculum was applied by blowing freshly collected conidia onto plants to minimize escapes. The disease reaction to *Erysiphe chicoracearum* was scored several times prior to the late flowering stage. Parents, F_1, and segregating families were scored by visually estimating the percentage of leaf surface covered by mildew.

Plants were assigned to the three following phenotypic classes: (i) *resistant* (R), $\leq$ 10% infection; (ii) *susceptible* (S), >10% to <80% infection; and (iii) *highly susceptible* (HS), $\geq$ 80% infection. The proportion of plants observed in each phenotypic class was compared with the ratio expected on the basis of appropriate genetic hypotheses. The goodness of fit to expected ratios was measured by the χ^2 statistic. Heterogeneity χ^2 for families within a cross was nonsignificant so that data for families within a cross were pooled for analysis.

Results and Discussion

The F_1 seed and embryo sets obtained from the cross *H. argophyllus* × HA 89 (0.59%, 2.1%, respectively) was lower than that from *H. debilis* × HA 89 (9.2%, 25.4%, respectively). However, the percentage of successfully rescued embryos was higher in crosses with *H. argophyllus* (Table 1).

We did not obtain F_2 seeds from the *H. debilis* × HA 89 cross, and only produced 17 BC_1F_1 plants from this cross using embryo rescue techniques (Rojas et al., 2003). The developed F_1 seeds were non-viable, probably due to embryo abortion. BC_1F_2 seeds had a germination rate of over 90%. In contrast, during the introgression of PM resistance from *H. argophyllus* into the cultivated sunflower, the seed set rate was higher in each generation and embryo rescue was only used for obtaining the F_1 plants (Table 1 & 2). Both F_1 and BC_1F_1 seed had over 90% germination. Therefore, *H. argophyllus* × HA 89 crosses had a higher percentage of viable F_1 seed than *H. debilis* × HA 89 crosses.

Although the F_1 progeny were evaluated in different seasons, we observed that only the F_1 plants of *H. argophyllus* were completely resistant to PM infection. The F_1 progeny from *H. argophyllus* × HA89 was likely to have a higher PM resistance than F_1 progeny of *H. debilis* × HA 89 (Table 3).

Table 1. Development of seeds and 5-day-old embryos and percent survival of cultured embryos from interspecific crosses of *H. argophyllus* and *H. debilis* and cultivated line HA 89.

Accession	No. plants	Developed seeds		Five-day-old embryos			
		No. heads	Seed set, % †	No. heads	Embryo set, % †	Embryos cultured ‡	Survival (%)
H. argophyllus	3	24	0.59§ (0.09-0.96)¶	24	2.1 (1.1-3.1)	77	40.6 (26.7-65.0)
H. debilis ssp. *debilis*	3	32	9.2 (4.3-12.4)	17	25.4 (6.8-61.1)	139	20.7 (17.1-26.8)

† Developed seeds or 5-day-old embryos per total pollinated flowers of each plant
‡ Using embryo rescue technique
§ Mean value
¶Range

Table 2. Seed set of *H. argophyllus* × HA 89 BC_1F_1, and *H. debilis* × HA 89 BC_1F_1 and BC_1F_2 pollinated by HA 89.

Accession	Generation	No. plants	Developed seeds	
			No. heads	Seed set, %
H. argophyllus	BC_1F_1	3	3	8.18 (5.9-9.6)
H. debilis ssp. *debilis*	BC_1F_1	3	3	0.15 (0-0.15)
	BC_1F_2	17	110	5.70 (0-33.1)

Table 3. Powdery mildew reaction of the F_1 progenies from crosses between resistant wild plants and the susceptible line HA 89.

Accession	PI	n†	Mean (range values) of infection (%)
H. argophyllus	494582	4	7.5 (5-10)
H. debilis ssp. *debilis*	435671	9	37 (10-100) ‡

† Number of plants; ‡ Score obtained during 2003

Previous studies suggested PM resistance from *H. debilis* was dominant or partially dominant (Rojas et al., 2003). The disease severity found in most resistant F_1 families in 2003 ranged from 10% to 30% of the leaf surface covered by mildew. In the summer 2004 we were

not able to obtain F_1 plants for direct comparisons to other generations. The BC_1F_2 families had disease ratings from 0% to 100%, suggesting no transgressive segregation (Table 5 and Fig. 1).

Table 4. Segregation of PM reaction in F_1 generation and BC_1F_1 families from a resistance accession of *H. argophyllus* and the susceptible line HA 89, and Chi-square (χ^2) tests.

Generation	No. of plants†			Tested ratio	χ^2	*P*-value
	R	S	HS			
H. argophyllus	5					
HA 89			5			
H. argophyllus× HA 89	4					
((F_1-1)×HA 89), BC_1F_1-2	3	12		1R:3S	0.20	0.65
((F_1-2)×HA 89), BC_1F_1-3	3	12		1R:3S	0.20	0.65
Pooled	6	24		1R:3S	0.40	0.53
Heterogeneity					0	1.00
((F_1-3)×HA 89), BC_1F_1-1	6	9		1R:1S	0.60	0.44

† R, resistant; S, susceptible; HS, highly susceptible

Table 5. Segregation of PM reactions and Chi-square (χ^2) tests of BC_1F_2 families from the cross between a resistance accession of *H. debilis* and the susceptible line HA 89.

Generation	No. of plants†			Tested ratio	χ^2	*P*-value
	R	S	HS			
H. debilis	5					
HA 89			5			
(F_1-2×HA 89), BC_1F_2-2	4	11	5	1R:2S:1HS	0.30	0.86
(F_1-1×HA 89), BC_1F_2-4		12	6	3S:1HS	0.0	1.00
(F_1-1×HA 89), BC_1F_2-1		5	12	1S:3HS	0.07	0.79
(F_1-2×HA 89), BC_1F_2-6		4	9	1S:3HS	0.23	0.63
Pooled		9	21	1S:3HS	0.24	0.62
Heterogeneity					0.06	0.81
(F_1-2×HA 89), BC_1F_2-3	8	8	3	9R:6S:1HS	3.50	0.17
(F_1-3×HA 89), BC_1F_2-5	8	5	2	9R:6S:1HS	1.08	0.58
Pooled	16	13	5	9R:6S:1HS	4.51	0.10
Heterogeneity					0.07	0.79

† R, resistant; S, susceptible; HS, highly susceptible

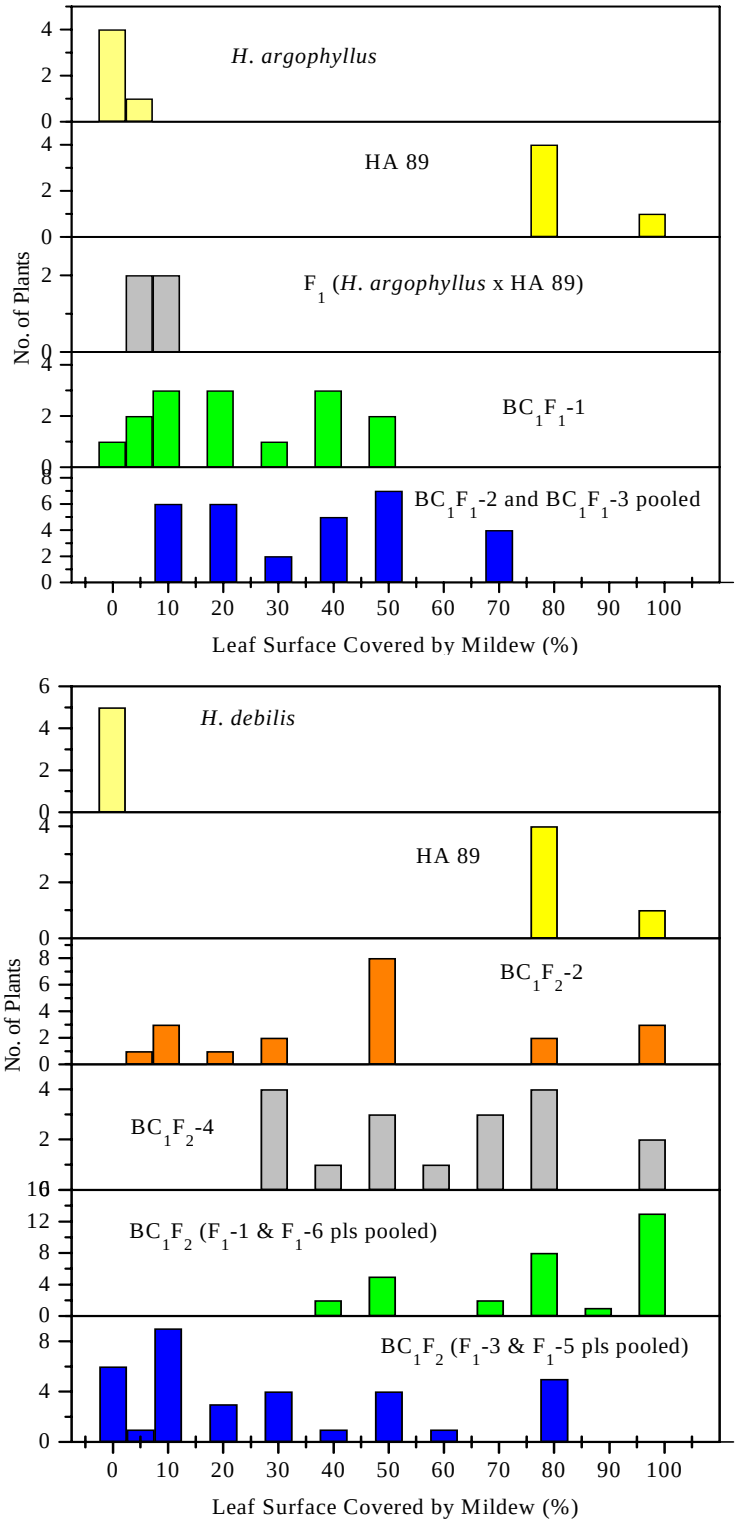

Figure 1. Frequency of distribution of PM reaction (*Erysiphe chicoracearum* D.C.) in wild resistant parents (*H. argophyllus* and *H. debilis* ssp. *debilis*), and the segregating progenies, F_1 and BC_1F_1 from the *H. argophyllus* × HA 89 cross, and BC_1F_2 from the *H. debilis* × HA 89 cross.

The leaves of *H. argophyllus* had 0% to 5% of the leaf surface covered by mildew, and F_1 plants showed 5% to 10% of infection, indicating that resistance was dominant (Table 4 and Fig. 1). The disease reaction of the BC_1F_1 plants ranged from 0% to 70% infection (Fig. 1). The BC_1F_1 families showed two different patterns of segregation which fit to the expected 1R: 1S ratio for one gene and the expected 1R: 3S ratio for two genes (Table 4 and Fig. 1). This difference between BC_1F_1 families indicates the wild parents were heterozygous for the resistance genes.

Similar to the BC_1F_1 families derived from *H. argophyllus* × HA 89, BC_1F_2 families derived from *H. debilis* × HA 89 also showed different patterns of segregation, confirming the heterozygosity of the wild parent for PM resistance genes. The segregation patterns in the BC_1F_2 families from *H. debilis* × HA 89 fit to the expected ratios of 1R:2S:1HS, 3S:1HS and 1S:3HS ratios for one gene, and for the expected 9R:6S:1HS ratio for two genes (Table 5).

The results from the *H. argophyllus* × HA 89 and *H. debilis* × HA 89 suggest that resistance in both crosses is controlled by at least two genes. The action of major genes in PM resistance inheritance from *H. debilis* ssp. *debilis* has been suggested previously (Jan and Chandler, 1985). Inheritance studies using an appropriate population size for polygenic inheritance are needed in order to determine the number of genes involved in PM resistance. On the other hand, although the low germination rates improved and the seed set increased with more backcrosses to the cultivated line in both interspecific crosses, the introgression of the PM resistance into cultivated sunflower appears to be easier using *H. argophyllus* than *H. debilis* ssp. *debilis.* This could be explained by the fact that *H. argophyllus* is a closer relative to cultivated sunflower. The resistant plant material obtained will be used for the development of new PM resistant germplasm.

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