Discovery of the Wild Sunflower-Derived Novel Downy Mildew Resistance Gene *Pl_{19}* in sunflower (*Helianthus annuus* L.)

Lili Qi[1], Zhiwei Zhang[2]

[1]USDA-ARS Northern Crop Science Laboratory, Fargo, ND, USA; [2]Department of Agronomy, Inner Mongolia Agricultural University, Inner Mongolia, China

Abstract

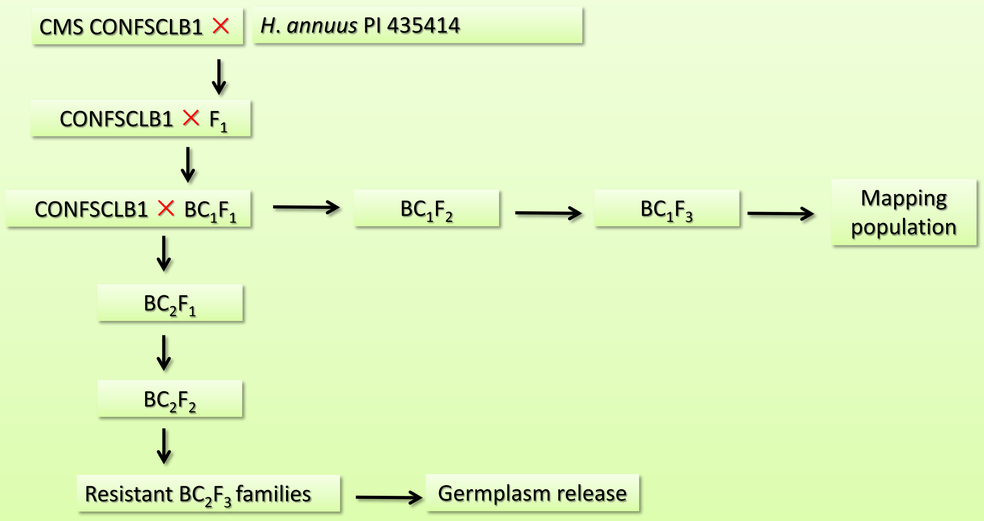
Wild *Helianthus annuus* accession PI 435414 exhibited resistance to downy mildew, which is one of the most destructive diseases to sunflower production globally. Evaluation of the 140 $BC_1F_{2:3}$ families derived from the cross of CMS CONFSCLB1 and PI 435414 against *Plasmopara halstedii* race 734 revealed that a single dominant gene controls downy mildew resistance in the population. Bulked segregant analysis conducted in the BC_1F_2 population with 860 simple sequence repeat (SSR) markers indicated that the resistance derived from wild *H. annuus* was associated with SSR markers located on linkage group (LG) 4 of the sunflower genome. To map and tag this resistance locus, designated *Pl_{19}*, 140 BC_1F_2 individuals were used to construct a linkage map of the gene region using both SSR and single nucleotide polymorphism (SNP) markers from LG4. Two flanking SNP markers, NSA_003564 and NSA_006089, were identified as surrounding the *Pl_{19}* gene at a distance of 0.6 cM from each side. Genetic analysis indicated that *Pl_{19}* is different from *Pl_{17}*, which had previously been mapped to LG4, but is closely linked to *Pl_{17}*. This new gene is highly effective against the most predominant and virulent races of *P. halstedii* currently identified in North America.

Introduction

Sunflower (*Helianthus annuus* L.) is adapted to a wide variety of soils and climatic conditions and is widely grown in the world. There are two basic types of sunflowers. Approximately 90 percent of world production is oil-type sunflower, which is used as a source of high quality vegetable oil. The remaining sunflowers are confection type, which is grown for human food consumption or bird feed. Downy mildew caused by *Plasmopara halstedii* is one of the most destructive diseases of sunflower worldwide. Historically, host genetic resistance has provided the most economic and environment friendly method for controlling downy mildew. However, the main drawback of using *R* genes to control resistance is that their effects are often not durable because of the rapidly evolving *P. halstedii* pathogen. This necessitates the continued search for new sources of resistance and designing new strategies for more durable resistance. In this research, we introgressed downy mildew resistance from wild *H. annuus* PI 435414 into cultivated sunflower and describe a new *Pl_{19}* gene that was discovered in wild *H. annuus* species and located in linkage group (LG) 4 of the sunflower genome.

Materials and Methods

Cross and backcross populations



Downy mildew evaluation

- A *P. halstedii* isolate of race 734was chosen to test seedlings of each backcross generation and the mapping population of the BC_1F_3 families for resistance to downy mildew.
- In addition, isolates of another five *P. halstedii* races, 314, 700, 710, 714, and 774, were selected to test the homozygous resistant BC_1F_3 families.
- Phenotypic variation was evaluated in the greenhouse trials. Sunflower seedlings infected by downy mildew display typical leaf chlorosis with white sporulation on the underside of cotyledons and true leaves.

Molecular mapping

- A total of 860 SSR markers were used to identify polymorphisms between the parents CONFSCLFB1 and PI 435414 of the mapping population. Bulked segregant analysis was applied to identify specific region associated with DM resistance.
- Once the *Pl* gene was positioned relative to the SSR markers, additional 27 SNP markers surrounding the region were selected from the three published sunflower SNP maps, which could be used to better define the genomic position of the *Pl* gene.

RESULTS

I. Transfer of downy mildew resistance from wild species *H. annuus* into cultivated sunflower

Initial cross was made between CMS line CONFSCLB1 with sunflower wild *H. annuus* accession PI 435414 in 2013. A novel downy mildew resistance gene, named *Pl_{19}*, was successfully transferred from the wild *H. annuus* to cultivated sunflower. *Pl_{19}* exhibits broad-spectrum resistance against *P. halstedii* races, 314, 700, 710, 714, 734, and 774, which are the most predominant and virulent races currently identified in North America and Europe (Table 1 and Fig. 1).

Table 1. Downy mildew multi-race tests of the homozygous BC_1F_3 family for resistance

Line	Downy mildew races											
	314		700		710		714		734		774	
	S	R	S	R	S	R	S	R	S	R	S	R
Cargill 270	24	0	27	0	22	0	20	0	27	0	22	0
HA-DM1	0	15	0	17	0	14	0	14	0	12	0	9
CONFSCLB1	31	0	28	0	29	0	29	0	31	0	25	0
14-213-69/BC_1F_3	0	37	0	37	0	38	0	39	0	37	0	33

Cargill 270, susceptible check; *HA-DM1,* resistant check; *CONFSCLB1,* susceptible recurrent parent; *14-213-69,* homozygous BC_1F_3 family; *S,* susceptible; *R,* resistant.

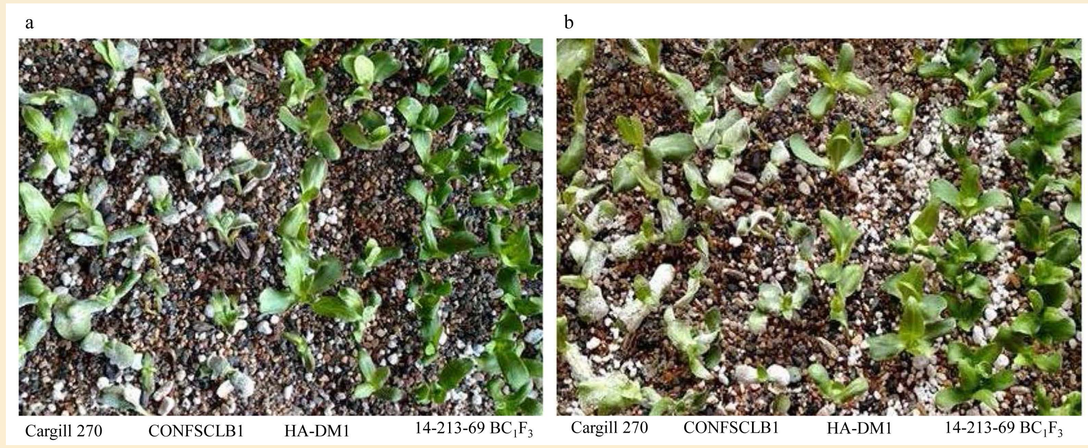


Fig 1. Downy mildew evaluation of the homozygous BC_1F_3 family of 14-213-69. a, *P. halstedii* race 710; b, *P. halstedii* race 734. Abundant white sporulation was observed on the underside of the leaf surface of Cargill 270 and CONFSCLB1, whereas no sporulation was noted on HA-DM1 and 14-213-69. Cargill 270, susceptible check; CONFSCLB1, susceptible recurrent parent; HA-DM1, resistance check; 14-213-69, homozygous BC_1F_3 family

II. Inheritance of downy mildew resistance in the mapping population

The parental line CONFSCLB1 and the 140 BC_1F_3 families (30 seedlings for each family) were inoculated with a *P. halstedii* isolate of race 734 in the greenhouse under controlled conditions. The segregation of the downy mildew resistance in the 140 $BC_1F_{2:3}$ families were 33 homozygous resistant, 78 segregating, and 29 homozygous susceptible plants which fits a 1 resistant: 2 heterozygous resistant: 1 susceptible segregation ratio (χ^2 = 2.0572, df = 2, *P* = 0.3575). The results indicated that a single dominant gene was responsible for downy mildew resistance in this population.

III. Molecular mapping of the downy mildew resistance gene from PI 435414

Bulked segregant analysis conducted in the BC_1F_2 population with 860 simple sequence repeat (SSR) markers indicated that the resistance derived from wild *H. annuus* was associated with SSR markers located on linkage group (LG) 4 of the sunflower genome. To map and tag this resistance locus, designated as *Pl_{19}*, 140 BC_1F_2 individuals were used to construct a linkage map of the gene region. Two SSR markers, ORS963 and HT298, were linked to *Pl_{19}* within a distance of 4.7 cM. Additional screening with 27 single nucleotide polymorphism (SNP) markers previously mapped to this genomic region revealed that SNP markers, NSA_003564 and NSA_006089, were flanking the *Pl_{19}* gene at a distance of 0.6 cM from each side (Fig. 2a). Genetic analysis indicated that *Pl_{19}* gene is different from *Pl_{17}* gene, which was previously mapped to the LG4, but is closely linked to *Pl_{17}* gene. In the *Pl_{19}* genetic map, the two linked common markers ORS963 and NSA_003564 were mapped upstream of *Pl_{19}* gene (Fig. 2a), while they were mapped downstream of *Pl_{17}* gene in the *Pl_{17}* genetic map (Fig. 2b).

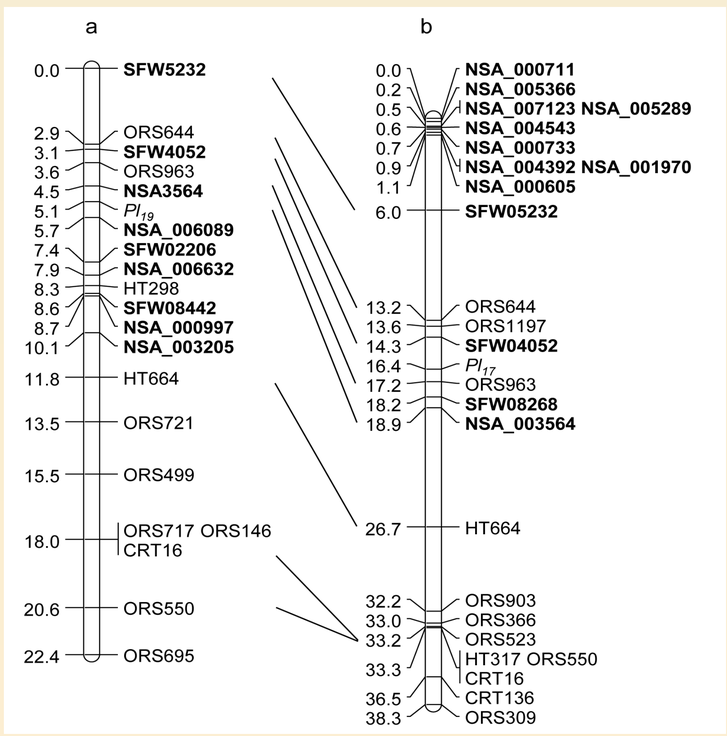


Fig 2. Genetic maps of sunflower linkage group (LG) 4. a, LG4 SSR and SNP combined map of *Pl_{19}*; b, LG4 *Pl_{17}* map taken from Qi et al. (2015)

References

Qi LL, Long YM, Jan CC, Ma GJ, Gulya TJ (2015) *Pl_{17}* is a novel gene independent of known downy mildew resistance genes in the cultivated sunflower (*Helianthus annuus* L.). Theor Appl Genet 128:757–767

Qi LL, Foley ME, Cai XW, Gulya TJ (2016) Genetics and mapping of a novel downy mildew resistance gene, *Pl_{18}*, introgressed from wild *Helianthus argophyllus* into cultivated sunflower (*Helianthus annuus* L.). Theor Appl Genet 129:741–752