Theor Appl Genet (2017) 130:29–39
DOI 10.1007/s00122-016-2786-z

ORIGINAL ARTICLE

Discovery and introgression of the wild sunflower-derived novel downy mildew resistance gene *Pl*$_{19}$ in confection sunflower (*Helianthus annuus* L.)

Z. W. Zhang[1] · G. J. Ma[2] · J. Zhao[1] · S. G. Markell[2] · L. L. Qi[3]

Received: 20 July 2016 / Accepted: 3 September 2016 / Published online: 27 September 2016

Abstract

Key message **A new downy mildew resistance gene, *Pl*$_{19}$, was identified from wild *Helianthus annuus* accession PI 435414, introduced to confection sunflower, and genetically mapped to linkage group 4 of the sunflower genome.**

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. Two SSR markers, ORS963 and HT298, were linked to *Pl*$_{19}$ within a distance of 4.7 cM. After screening 27 additional single nucleotide polymorphism (SNP) markers previously mapped to this region, 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 and is the first downy mildew resistance gene that has been transferred to confection sunflower. The selected resistant germplasm derived from homozygous BC_2F_3 progeny provides a novel gene for use in confection sunflower breeding programs.

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 % of world production is oil-type sunflower, which is used as a source of high-quality vegetable oil. The remaining sunflowers grown are the confection type, which is grown for human food consumption or bird feed (Hladni 2016). Breeding for confection sunflower is different from that of oil type, focusing on large seeds as well as increased test weight, protein content and quality. The seed of confection sunflower with white striped hulls is larger than that of the oilseed types and has a lower oil content and test weight. The United States (US) is a major global producer of confection sunflower, supplying both domestic needs and export markets. The use of sunflower

Communicated by V. Hahn.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-016-2786-z) contains supplementary material, which is available to authorized users.

✉ L. L. Qi
lili.qi@ars.usda.gov

[1] Department of Agronomy, Inner Mongolia Agricultural University, Huhhot 010019, Inner Mongolia, China

[2] Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA

[3] Northern Crop Science Laboratory, USDA-Agricultural Research Service, 1605 Albrecht Blvd. N, Fargo, ND 58102-2765, USA

seed in human diets as a snack or for birdfeed has grown consistently over the past 15 years (US National Sunflower Association 2011).

Downy mildew caused by *Plasmopara halstedii* (Farl.) Berl. & de Toni is a yield-limiting disease that occurs in all areas of intensive sunflower production throughout the world, except Australia (Markell et al. 2015). *P. halstedii* overwinters as a thick-walled oospore that can survive many years in the soil. Germinated oospores produce zoosporangia which release motile zoospores that infect germinating seedlings, causing a systemic infection that kills most infected plants (Harveson et al. 2016). Infected plants that survive produce a sterile head and act as a competitor with adjacent plants in the field (Markell et al. 2015). Thus, 100 % yield loss of each systemically infected plant is expected. Downy mildew is not observed uniformly throughout fields; rather, it occurs in areas of fields prone to saturated soils (for example, low spots). As a result, nearly 100 % yield loss can occur in large patches of sunflower fields when environmental conditions are favorable for infection (Gulya et al. 2013). The frequency of fields infected by downy mildew in a region depends heavily on rainfall after planting, but can be very high. In the US state of North Dakota, which frequently leads the country in production, the prevalence of sunflower fields infected with downy mildew ranged from less than 2 % to over 70 % between 2001 and 2011, with a mean prevalence of 33.9 % over those 11 years (Gulya et al. 2013).

Confection sunflower is more vulnerable to downy mildew than oil sunflower due to a lack of resistance sources. The *P. halstedii* pathogen was first identified in North America (Nishimura 1922; Young and Morris 1927) and was later reported in Russia and Western Europe in approximately 1960 (Novotelnova 1960). In the 1970s, two *P. halstedii* races were identified, one each in Europe and North America (Zimmer 1974). To combat this destructive disease, host race-specific resistance genes (*R* genes), denoted as *Pl*, were deployed at a large scale in sunflower production, especially in the late 1990s when the *P. halstedii* population developed resistance to the widely used seed treatment mefenoxam (Albourie et al. 1998; Gulya et al. 1999, 2013). However, over 40 years, the *P. halstedii* pathogen has constantly evolved new physiological races due to its pathogenic variability and the selection pressure resulting from the use of resistant hybrids in sunflower production. To date, at least 24 *P. halstedii* races have been defined in Europe and 40 in the Americas (Gulya et al. 2011; Gascuel et al. 2015; Viranyi et al. 2015), posing a serious threat to global sunflower production. Gilley et al. (2016) screened 14 downy mildew-resistant lines using 185 isolates collected from US sunflower fields in 2014 and 2015, and found that only a few lines were resistant to all isolates, none of which contained known resistance genes present in confection sunflower. Thus, continuing the search for new *Pl* genes that provide resistance to new *P. halstedii* strains is a critical task for sunflower researchers to protect sunflower production from the yield loss that occurs from downy mildew.

The cultivated sunflower ($2n = 2x = 34$) belongs to the *Helianthus* genus, a member of the *Compositae* (*Asteraceae*) family, which is the largest and most diverse family of flowering plants (Funk et al. 2005). The *Helianthus* genus contains 53 wild species comprising 14 annual and 39 perennial species (Schilling 2006; Stebbins et al. 2013). In the last four decades, a total of 1359 annual accessions and 842 perennial accessions were collected in the US (Seiler and Marek 2014). These provide many useful genes for sunflower improvement, such as better quality and higher yield, cytoplasm male sterility, disease resistance and tolerance for biotic and abiotic stresses (Seiler and Jan 2010). Sunflower wild species are also an important source of downy mildew resistance genes. Fourteen *Pl* genes (Pl_1, Pl_2, Pl_5–Pl_8, Pl_{13}–Pl_{18}, Pl_{21}, and Pl_{Arg}) that were previously mapped to the sunflower genome can be traced to their wild origin, most of which were derived from wild annual species, especially *H. annuus* (Miller and Gulya 1987, 1988, 1991; Seiler 1991, 2010; Vear et al. 2008; Mulpuri et al. 2009; Hulke et al. 2010; Qi et al. 2016). This species contributed five of the 14 *Pl* genes (Pl_1, Pl_2, Pl_6, Pl_{13}, and Pl_{17}) and is considered to be the most valuable source of *Pl* genes. Gulya (2005) screened an additional 286 accessions of the wild *H. annuus* using the most virulent *P. halstedii* race at the time (race 773) and found 26 accessions where >90 % plants displayed immunity to systemic infection. Among the 14 wild annual species, which are all diploid with 34 chromosomes, the same number as cultivated sunflower, *H. annuus* is the most diverse species of sunflower in North America. This species is found throughout the US, but is more common in the western two-thirds of the country (Rogers et al. 1982; Kantar et al. 2015). However, most of the resistant accessions identified by Gulya (2005) originated from the south central US, with a high amount of them originating from the state of Texas.

The wild *H. annuus* accession PI 435414 was originally collected from Paris, Texas, US in 1978. This accession was first identified to be resistant to downy mildew by Gulya (2005) and further confirmed by Markell and Humann in 2013 (personal communication). In this research, we introgressed downy mildew resistance from PI 435414 into confection sunflower and describe a new *Pl* gene that was discovered in wild *H. annuus* species and located in linkage group (LG) 4 of the sunflower genome. This gene, designated Pl_{19}, confers resistance to several new virulent races of *P. halstedii* currently identified in North America, providing a novel source for use in confection sunflower breeding programs.

Materials and methods

Cross and backcross populations

Due to open-pollinated nature, wild *H. annuus* accession PI 435414 is segregating for downy mildew resistance. PI 435414 plants were screened with *P. halstedii* race 734, and the selected resistant plants were crossed with the cytoplasmic male sterile (CMS) CONFSCLB1 in May 2013. The selected F_1-resistant plants were backcrossed twice to the maintainer CONFSCLB1. CONFSCLB1 is a confection BC_1F_3-derived BC_1F_4 maintainer selected from the cross of HA 441/ROM PH//HA 442/HA 441/4/CONF/5/CONF and is susceptible to downy mildew. The line was released by the USDA-ARS Sunflower Research Unit and the North Dakota Agricultural Experiment Station in 2006. The F_1 and each of backcross generations were screened in the greenhouse for downy mildew resistance to *P. halstedii* race 734 as described below. The selected downy mildew-resistant BC_2F_1 plants were advanced to the BC_2F_2 generation.

Mapping population

For molecular mapping of the downy mildew resistance gene derived from PI 435414, the BC_1F_2 and BC_1F_2-derived BC_1F_3 populations were developed from a single resistant BC_1F_1 plant from the cross of CMS CONFSCLB1 and PI 435414. The BC_1F_2 individuals were grown in a greenhouse in the winter of 2014–2015 and were self-pollinated to produce $BC_1F_{2:3}$ families that were used in subsequent disease evaluation.

Downy mildew resistance evaluation

A *Plasmopara halstedii* isolate of race 734, which was first identified in 2009 in North America and overcame the Pl_6 and Pl_7 genes (Gulya et al. 2011), was chosen to test seedlings of each backcross generation and the mapping population of the BC_1F_3 families for resistance to downy mildew. Phenotypic variation was evaluated in greenhouse trials using the whole seedling immersion technique described by Gulya et al. (1999) and Qi et al. (2015). Sunflower seedlings infected by downy mildew display typical leaf chlorosis with white sporulation on the underside of cotyledons and true leaves. A plant was considered to be susceptible (S) if sporulation was observed on cotyledons and true leaves and was considered to be resistant (R) if no sporulation was observed. In addition, isolates of another five *P. halstedii* races, 314, 700, 710, 714, and 774, which are the most predominant and highly virulent races in North America and Europe (Gulya et al. 2011; Gascuel et al. 2015; Gilley et al. 2016), were selected to test the homozygous resistant BC_1F_3 families derived from the cross of CONFSCLB1 and PI 435414 and the susceptible recurrent parent CONFSCLB1 with Cargill 270 and HA-DM1 carrying the Pl_{18} gene as susceptible and resistant controls, respectively.

Artificial infection of $BC_1F_{2:3}$ seedlings was used to determine the segregation of the downy mildew *R* gene in the BC_1F_2 population. A total of 40 seeds from each 140 $BC_1F_{2:3}$ families were germinated at 22–24 °C in a growth chamber, and 30 seedlings of each family were inoculated with a *P. halstedii* isolate of race 734. The BC_1F_3 families were classified as homozygous resistant if none of the seedlings had sporulation, segregating if some seedlings (about one-quarter in an F_3 family) had sporulation on the cotyledons and true leaves, and homozygous susceptible if all seedlings had sporulation on cotyledons and true leaves, which represented the genotypes of downy mildew resistance in each F_2 individual.

DNA extraction and SSR PCR condition

Young leaf tissues from the parents, CONFSCLB1 and PI 435414, and 140 BC_1F_2 plants were collected and freeze-dried. Tissues samples were also collected from the BC_2F_2 population. DNA extraction from lyophilized tissues was performed using a DNeasy 96 plant kit following the manufacturer's instructions (Qiagen, Valencia, CA, USA). The quantity and quality of DNA were determined using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The concentration of DNA was adjusted to 5 ng/μl for PCR amplification.

For the simple sequence repeat (SSR) marker, 10–20 ng of template DNA was added to a 15-μl polymerase chain reaction (PCR) mixture, including 0.75 μl of 50 mM $MgCl_2$, 1.5 μl of 2.5 mM dNTPs, 0.9 μl of 10× PVP-40, 1.5 μl of 0.2 pmol of the forward primer with a M13 tail (CACGACGTTGTAAAACGAC) at the 5′ end, 1.5 μl of 1.0 pmol of the reverse primer, 1.5 μl of 1.0 pmol of the fluorescently labeled M13 primer, 1.5 μl of 10× PCR buffer, and 0.5 units of Taq polymerase (Bioline, Randolph, MA, USA). PCR was performed in a Peltier thermocycler (Bio-Rad Lab, Hercules, CA, USA) using the touchdown program as described by Qi et al. (2011). PCR products were detected by an IR2 4300/4200 DNA Analyzer with denaturing polyacrylamide gel electrophoresis (LI-COR, Lincoln, NE, USA).

Bulked segregant analysis and genetic mapping

A total of 860 SSR markers were used to identify polymorphisms between the parents CONFSCLB1 and PI 435414 of the mapping population. For bulked segregant analysis (BSA) (Michelmore et al. 1991), genomic DNA from BC_1F_2 of ten homozygous resistant plants and ten

Table 1 The primer sequences of the SNPs mapped in this study

SNP name	SNP primer	Primer sequence (5′–3′)
SFW05232	SFW05232F1	GCAACAGGAACCAGCTATGACCCGAACCTATGTAACGTAG
	SFW05232F2	GCAACAGGAACCAGCTATGAC*ATGAC*CCGAACCTATGTAACACAA
	SFW05232R	TGCATACGAAAGATGCGTCC
SFW04052	SFW04052F1	GCAACAGGAACCAGCTATGACCCAATTGTGTTGGTTTTAACT
	SFW04052F2	GCAACAGGAACCAGCTATGAC*ATGAC*CCAATTGTGTTGGTTTTGGCC
	SFW04052R	TGTTGTATTTGAACCGCCAGT
SFW08442	SFW08442F1	GCAACAGGAACCAGCTATGACGATCGCCAATTTACCATTTTCTTCA
	SFW08442F2	CAACAGGAACCAGCTATGAC*ATGAC*GATCGCCAATTTACCATTTTCCCCC
	SFW08442R	GGAAAAAGCGTGTTTGTGAACC
SFW02206	SFW02206F1	GCAACAGGAACCAGCTATGACCGGTGAAGTATGCCGGCATT
	SFW02206F2	CAACAGGAACCAGCTATGAC*ATGAC*CGGTGAAGTATGCCGGACTC
	SFW02206R	CGTCGGTTCTAACGCTTTGC
NSA_003564	NSA_003564F1	GCAACAGGAACCAGCTATGACTTTCAGCTACCGTACAGCAAAAA
	NSA_003564F2	GCAACAGGAACCAGCTATGAC*ATGAC*TTTCAGCTACCGTACAGCAGGAG
	NSA_003564R	TCCGGTTCTCAAATGACCCA
NSA_006089	NSA_006089F1	GCAACAGGAACCAGCTATGACGCTCCTCAAATAATTCAGCCTTTTATTCTA
	NSA_006089F2	GCAACAGGAACCAGCTATGAC*ATGAC*GCTCCTCAAATAATTCAGCCTTTTATTCTAG
	NSA_006089R	GGGTCACTAGTAGCCCAAAGT
NSA_006632	NSA_006632F1	GCAACAGGAACCAGCTATGACGAATAAGAACTATCTAGCAAGATGCTCAA
	NSA_006632F2	GCAACAGGAACCAGCTATGAC*ATGAC*GAATAAGAACTATCTAGCAAGATGCCAAG
	NSA_006632R	AACCAAGTGAAGCAGATATCGAGT
NSA_000997	NSA_000997F1	GCAACAGGAACCAGCTATGACGTTGTCGGCAGTGTATTTTTGCCA
	NSA_000997F2	GCAACAGGAACCAGCTATGAC*ATGAC*GTTGTCGGCAGTGTATTTTTAACC
	NSA_000997R	GAGGTGCCAAAATAGGTGGGT
NSA_003205	NSA_003205F1	GCAACAGGAACCAGCTATGACCTACCATTGTATCGGCACACACG
	NSA_003205F2	GCAACAGGAACCAGCTATGAC*ATGAC*CTACCATTGTATCGGCACAACCA
	NSA_003205R	CCCAACTACCACGTTCGCTT

The tail sequence is underlined, and the additional five-base oligonucleotide insertion in AS-primer F2 is italicized

homozygous susceptible plants were pooled in equal proportions to create the resistant and susceptible bulks. SSR markers that were polymorphic between the parents were used to test the S- and R-bulks. Once the potential locations of resistance gene were identified, additional markers from these locations were used to genotype the population.

SSR marker and downy mildew phenotype data were used to construct a genetic map of the 140 BC_1F_2 individuals from the CONFSCLB1 × PI 435414 population. Linkage analysis was performed using JoinMap 4.0 with a regression mapping algorithm and Kosambi's mapping function (Van Ooijen 2006). The Chi-square (χ^2) test was used to assess the goodness of fit to the expected segregation ratio for each marker. A minimum likelihood of odds (LOD) ≥3.0 and a maximum distance of ≤50 centimorgans (cM) were used to test linkages among markers.

SNP marker and genotyping

Once the *Pl* gene was positioned relative to the SSR markers, additional 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. Thirteen SNPs were SFW SNPs (Bowers et al. 2012) covering the region from 5.21 to 20.78 cM, and 14 SNPs were NSA SNPs (Talukder et al. 2014; Hulke et al. 2015) covering the region from 11.04 to 21.01 cM on LG4 (Table S1) in the respective maps.

Genotyping of SNPs was performed using a strategy of converting the SNPs into length polymorphism markers as described by Qi et al. (2016). Four primers were used in each PCR: a priming-element-adjustable primer (PEA-primer 5′-ATAGCTGG-Sp9-GCAACAGGAACCAGCTATGAC-3′) with an attached fluorescence tag at the 5′ terminus as a universal primer, two-tailed forward allele-specific primers (AS-primers F1 and F2, the tail sequences were identical to the PEA-primer), and one common reverse primer. An additional 5-base oligonucleotide (5′-ATGAC-3′) was inserted between the tail and allele-specific sequences in the AS-primer F2 to produce a length difference between the two SNP alleles after amplification (Table 1). The primers were screened for polymorphisms among the parents and BC_1F_2 population using the methods described by Qi et al. (2016). PCR products were detected by an IR2 4300/4200 DNA Analyzer with denaturing polyacrylamide gel electrophoresis (LI-COR, Lincoln, NE, USA).

Results

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

Confection sunflower of the cytoplasmic male sterile (CMS) line CONFSCLB1 was used as the female in an initial cross with downy mildew-resistant plants selected from *H. annuus* accession PI 435414 in May 2013. Not all of the F_1 plants were resistant given that the wild species accessions were not homozygous for genes controlling downy mildew resistance because they have an open-pollinated nature. A total of 50 F_1 plants were tested for resistance against *P. halstedii* race 734. Forty-two F_1 plants were resistant, and eight were susceptible. The selected F_1 resistant plants were used as male parents backcross to CONFSCLB1 with a normal cytoplasm in December 2013. The downy mildew test of the 82 BC_1 plants against race 734 in April 2014 indicated that 46 BC_1 plants were resistant and 36 were susceptible, fitting an expected 1:1 segregation ratio of the BC_1 population ($\chi^2 = 1.2195$, $df = 1$, $P = 0.2695$). The resistant BC_1 plants were used again as the male parent and were crossed to the recurrent parent CONFSCLB1 to produce the BC_2 generation. After a test of the BC_2 seedlings, the selected resistant BC_2 plants were self-pollinated to produce the BC_2F_2 population for the further selection of homozygous resistant plants.

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 a greenhouse under controlled conditions in June 2015. White sporulation of *P. halstedii* was clearly detected on the cotyledons and true leaves of CONFSCLB1. The susceptible seedlings in the BC_1F_3 population exhibited similar symptoms to those of CONFSCLB1, whereas the resistant seedlings had no sporulation. The segregation of the downy mildew resistance for the 140 $BC_1F_{2:3}$ families was 33 homozygous resistant, 78 segregating, and 29 homozygous susceptible plants and fits a 1 resistant:2 heterozygous resistant:1 susceptible segregation ratio ($\chi^2 = 2.0572$, df = 2, $P = 0.3575$). The results indicated that a single dominant gene was responsible for downy mildew resistance in the PI 435414 population.

Molecular mapping of the downy mildew gene from PI 435414

Over 860 SSR markers previously mapped to the sunflower genome (Tang et al. 2002; Yu et al. 2003) were used to screen polymorphisms between two parental lines, CONFSCLB1 and PI 435414, and approximately 43 % of the SSR markers were polymorphic between the two parents. Subsequent bulked segregant analysis (BSA) (Michelmore et al. 1991) was conducted between resistant and susceptible bulks with the all of the polymorphic SSR markers. BSA analysis revealed that the downy mildew resistance from PI 435414 was associated with the SSR markers HT664, ORS499, ORS721, and ORS963 from LG4. These four SSRs plus seven additional polymorphic LG4 SSRs were then used to genotype the 140 individuals of the BC_1F_2 population. Among the 11 SSR markers tested, eight co-dominant SSRs segregated at the expected 1:2:1 ratio, whereas three dominant SSRs, ORS963, HT664, and ORS721, segregated at the expected 3:1 ratio. The resistant locus from PI 435414, designated Pl_{19}, was mapped 1.5 cM from ORS963 and 3.2 cM from HT298 in a 4.7-cM interval in LG4 (Fig. 1a).

Marker enrichment in the Pl_{19} region

To identify additional markers in the Pl_{19} region, a total of 27 SNP markers located on LG4 were used to saturate the Pl_{19} genetic map (Bowers et al. 2012; Talukder et al. 2014; Hulke et al. 2015). After screening these SNP markers between the two parents, CONFSCLB1 and PI 435414, nine SNPs were identified as polymorphic and were subsequently evaluated across the entire BC_1F_2 population. No segregation distortion was identified for these SNP markers in the population, and the resulting map placed nine SNP markers at an interval of 10.4 cM in the upper end of the LG4 genetic map, encompassing the Pl_{19} locus (Fig. 1a). This mapping narrowed the resistance gene Pl_{19} from a 4.7-cM to a 1.2-cM region on LG4, flanked on one side by the SNP marker NSA_003564 and on the other side by NSA_006089 (Fig. 1a).

Physical linkage analysis of the clustered Pl_{19} and Pl_{17} in LG4

Pl_{19} is the second downy mildew *R* gene that mapped to LG4 of the sunflower genome and showed a close linkage to the Pl_{17} gene (Fig. 1, Qi et al. 2015). In the Pl_{19} and Pl_{17} genetic maps, the common markers ORS963 and NSA_003564 were mapped downstream of Pl_{17} (Fig. 1b) but upstream of Pl_{19} (Fig. 1a). The SSR marker ORS963 is the nearest marker that is closely linked to Pl_{17} and had different PCR pattern between PI 435414 and HA 458 (Pl_{17} donor in mapping population). Among five DNA markers, ORS1197, SFW04052, ORS963, SFW08268, and NSA_003564 surrounding Pl_{17}, ORS1197 and SFW08268 did not have a polymorphism in the Pl_{19} population. The SNP marker NSA_006089 closely linked to Pl_{19} was also not mapped to the Pl_{17} population (Fig. 1, Qi et al.

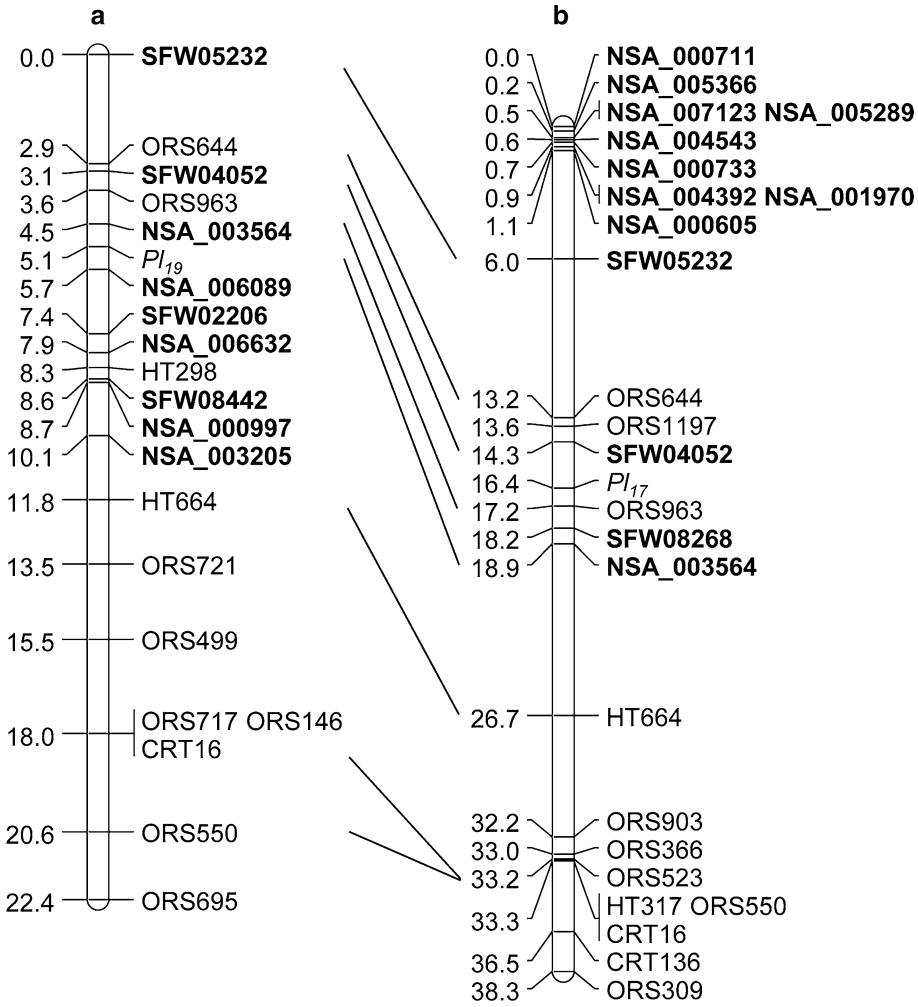

Fig. 1 Genetic maps of sunflower linkage group (LG) 4. **a** LG4 SSR and SNP combined map of Pl_{19}; **b** LG4 Pl_{17} map taken from Qi et al. (2015)

2015). These results indicated that Pl_{19} and Pl_{17} are different genes, but are closely linked to each other as a gene cluster.

Aligning ten SNPs sequences against the whole sunflower genome of HA412 v1.1. pseudomolecules available at http://sunflowergenome.org/early_access/repository/main/pseudomolecules/ indicated that the two genes are physically located at an interval of 3231,660 bp at the upper end of LG4 (Table 2). Pl_{17} is located between 3,621,089 and 6,813,151 bp, whereas Pl_{19} is likely located between 6,813,151 and 6,852,749 bp. The genetic order of eight SNP markers were consistent with their physical position, whereas two SNPs, NSA_03564 and SFW02206, were slightly different regarding their genetic and physical order (Table 2; Fig. 1).

Spectrum of downy mildew resistance from PI 435414

A BC_1F_3 homozygous *R*-family, 14-213-69, was selected to test against additional five *P. halstedii* races, 314, 700, 710, 714, and 774, along with race 734, which represents the most predominant and virulent races currently identified in North America (Gulya et al. 2011; Gilley et al. 2016). Seedlings of 14-213-69 along with susceptible and resistant checks, Cargill 270 and HA-DM1, respectively, and the susceptible recurrent parent CONFSCLB1 were inoculated with an isolate of each race separately. Cargill 270 is a hybrid of one of the nine standard differential lines used to identify *P. halstedii* races and is susceptible to all races. HA-DM1 is a recently released downy mildew-resistant line harboring the Pl_{18} gene and is resistant to all NA races (Gilley et al. 2016; Qi et al. 2016; Qi and Seiler 2016). As expected, Cargill 270 and CONFSCLB1 were susceptible to all of the races tested, and abundant white sporulation was observed on the leaf surface of their seedlings. In contrast, HA-DM1 and 14-213-69 were immune to all of the races, with no sporulation on cotyledons and true leaves (Fig. 2; Table 3), indicating that the resistance from PI 435414 has a broad spectrum of activity against downy mildew.

Table 2 Comparison of the physical position of Pl_{19} and Pl_{17} in the sunflower genome

SNP	SFW map[a] (cM)	NSA map[b] (cM)	Pl_{17} map[c]	Pl_{19} map	HA412.v1.1.bronze. 20141015 pseudomolecules			
					Name	Size	Start	End
SFW05232	5.21		6.0	0.0	Ha4	216,026,857	1,829,722	1,829,842
SFW04052	12.10		14.3	3.1	Ha4	216,026,857	3,621,089	3,621,181
Pl_{17}			16.4		Ha4			
SFW08268	15.62		18.2		Ha4	216,026,857	6,813,054	6,813,151
NSA_003564		13.860	18.9	4.5	Ha4	216,026,857	7,625,174	7,625,463
Pl_{19}				5.1	Ha4			
NSA_006089		13.058		5.7	Ha4	216,026,857	6,852,417	6,852,749
NSA_006632		–		7.9	Ha4	216,026,857	8,563,589	8,563,956
NSA_000997		15.119		8.7	Ha4	216,026,857	8,569,392	8,569,799
SFW08442	19.602			8.6	Ha4	216,026,857	8,623,438	8,623,558
SFW02206	19.784			7.4	Ha4	216,026,857	8,837,009	8,837,128
NSA_003205		19.867		10.1	Ha4	216,026,857	10,338,421	10,338,781

[a] Taken from Bowers et al. (2012)

[b] Taken from Talukder et al. (2014)

[c] Taken from Qi et al. (2015)

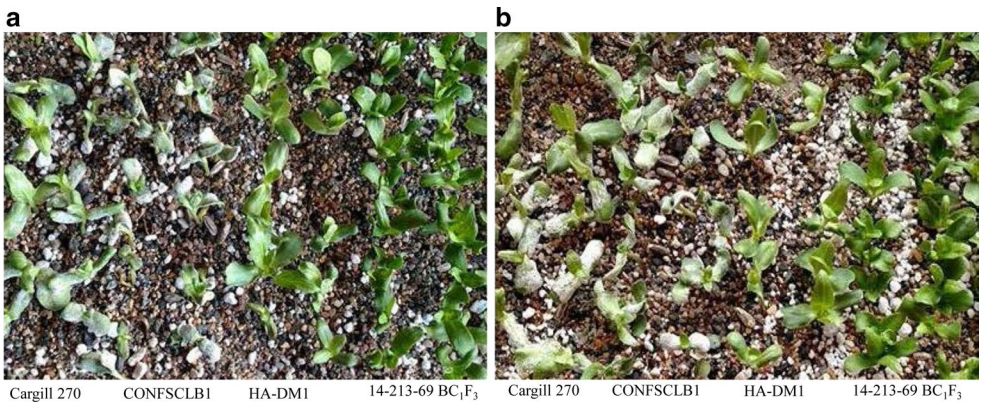

Fig. 2 Downy mildew evaluation of the homozygous BC_1F_3 family of 14-213-69. **a** *P. halstedii* race 710; **b** *P. halstedii* race 734. Abundant white sporulation was observed on the underside of the leaf surface of Cargill 270 and CONFSCLB1, whereas no sporulation was noted on HA-DM1 and 14-213-69. Cargill 270, susceptible check; CONFSCLB1, susceptible recurrent parent; HA-DM1, resistance check; 14-213-69, homozygous BC_1F_3 family

Table 3 Downy mildew multirace 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		27		22		20		27		22	
HA-DM1		15		17		14		14		12		9
CONFSCLB1	31		28		29		29		31		25	
14-213-69/BC_1F_3		37		37		38		39		37		33

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

Marker selection of the homozygous resistant plants from the BC_2F_2 population

To select homozygous resistant plants from the BC_2F_2 population of a CONFSCLB1/PI 435414 cross, two SNPs, SFW04052 and NSA_006089, were initially used to screen 94 BC_2F_2 individuals. Eighteen BC_2F_2 plants were identified as homozygous plants with identical PI 435414 marker alleles and were tested with the three additional markers, ORS963, NSA_03564, and SFW02206, which are closely linked to Pl_{19}. As anticipated, only the PI 435414 PCR patterns of the tested markers were detected in the homozygous BC_2F_2 plants, which were advanced to the BC_2F_3 generation. To further confirm the marker–trait association, a total of 185 $BC_2F_{2:3}$ progenies derived from the putative homozygous resistance of BC_2F_2 selected by DNA markers were inoculated with race 734. All of the BC_2F_3 plants exhibited resistance to downy mildew, and no segregation was observed. These findings confirmed the correlation of the phenotype and genotype, indicating that these markers were associated with the downy mildew resistance derived from PI 435414.

Discussion

Downy mildew is one of the most damaging diseases for sunflower production in North America and Europe as well as Asia and South America. Historically, host genetic resistance has provided an economically and environmentally 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 due to the rapid evolution of *P. halstedii*. This limitation necessitates the continued search for new sources of resistance and the design of new strategies for more durable resistance. In this study, downy mildew resistance was successfully transferred from the wild *H. annuus* accession PI 435414 to confection sunflower. Seedling resistance to *P. halstedii* race 734 in the segregation population was conferred by a single dominant gene, designated Pl_{19}. This gene was mapped to LG4 of the sunflower genome and flanked by two SNP markers, NSA_003564 and NSA_006089, in a 1.2-cM interval. These markers are suitable for marker-assisted selection, especially if Pl_{19} can be used in combination with other *Pl* genes. In addition, 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 (Gulya et al. 2011; Gascuel et al. 2015; Gilley et al. 2016). To the best of our knowledge, this is the first downy mildew gene in the confection sunflower background, and the selected homozygous BC_2F_3 germplasm will be extremely useful to develop downy mildew-resistant confection hybrids for commercial production.

As the second *Pl* gene mapped to LG4, Pl_{19} is located in a region that is closely linked to the downy mildew *R* gene Pl_{17}. Both Pl_{19} and Pl_{17} originate from wild *H. annuus* of different accessions, PI 435414 and PI 468435, respectively. The former was collected from the US state of Texas, and latter was obtained from the US state of Idaho. (https://npgsweb.ars-grin.gov/gringlobal/search.aspx). Based on the genetic linkage analysis, Pl_{19} is located downstream of Pl_{17} and is separated by the common markers ORS963 and NSA_003564 in the order SFW04052-Pl_{17}-ORS963/NSA_003564-Pl_{19}-NSA_006089 (Fig. 1). The alleles amplified from Pl_{19} and Pl_{17} for the common marker ORS963 were clearly discriminated according to their PCR pattern and fragment size (data not shown). ORS963 segregated as a co-dominant marker in the Pl_{17} population (Qi et al. 2015) and exhibited a dominant nature in the Pl_{19} population. Additionally, the SNP marker NSA_006089 nearest to Pl_{19} was not mapped to the Pl_{17} population, suggesting a null allele or altered priming site. These results indicate that Pl_{19} is a novel downy mildew *R* gene that is different from Pl_{17}, and ongoing allelism test efforts will help further elucidate the relationships of these two genes. Sequence alignments of SFW04052 and NSA_006089 flanking Pl_{17} and Pl_{19} against sunflower whole genome sequences indicated that Pl_{17} and Pl_{19} are physically located at an interval of 3,231,660 bp in the upper end of LG4 (Table 2), where the recombination frequency of 0.59 Mb/cM is fourfold higher than the genomic average of 2.4 Mb/cM, making it a region that is amenable to map-based cloning of genes of interest (Talukder et al. 2014; Qi et al. 2015). The closest flanking markers can be used as starting points for the positional cloning of Pl_{17} and Pl_{19} in the future to further elucidate the genetic mechanism underlining this gene cluster.

Sunflower wild species of *H. annuus* are an important source of downy mildew resistance and other disease resistances (for review see Seiler and Jan 2010). Among 15 mapped downy mildew *Pl* genes (Pl_1, Pl_2, Pl_5–Pl_8, Pl_{13}–Pl_{19}, Pl_{21}, and Pl_{Arg}), six (Pl_1, Pl_2, Pl_6, Pl_{13}, Pl_{17}, and Pl_{19}) are known to originate from the wild *H. annuus* (Fick and Zimmer 1974; Miller and Gulya 1991; Vear et al. 2008). These *Pl* genes from *H. annuus* tend to appear as a gene cluster in the sunflower genome. Pl_1, Pl_2, and Pl_6 are clustered on LG8 (Bouzidi et al. 2002; Slabaugh et al. 2003). Pl_{13} is part of a cluster on LG1 (Mulpuri et al. 2009; Liu et al. 2012), and Pl_{19}, mapped in the current study, is in a cluster with Pl_{17} on LG4 (Qi et al. 2015). Interestingly, three *Pl* gene clusters plus another *Pl* gene cluster on LG13 are all located on the distal regions of sunflower chromosomes (Slabaugh et al. 2003; Bachlava, et al. 2011; Liu et al. 2012; Qi et al. 2015). The gene clusters on LGs 1 (Pl_{13}, Pl_{14}, and Pl_{16}) and 13 (Pl_5, Pl_8, and Pl_{21}) are in the

lower end of the chromosomes, whereas the clusters on LGs 4 (Pl_{17} and Pl_{19}) and 8 (Pl_1, Pl_2, Pl_6, P_{l7}, and Pl_{15}) are in the upper end of the chromosomes. The only two *Pl* genes that are not in the cluster, Pl_{Arg} and Pl_{18}, are located in the middle of the one arm of sunflower chromosomes 1 and 2, respectively (Wieckhorst et al. 2010; Qi et al. 2016). The evolution of the *R* gene clusters has been extensively investigated in *Arabidopsis* (Kunkel 1996; Leister 2004), flax (Ellis et al. 1995, 1997, 2000), lettuce (Kesseli et al. 1994; Michelmore and Meyers 1998), tomato (Jones et al. 1993; Kaloshian et al. 1995; Takken et al. 1999), rice (Song et al. 1995, 1997; Wang et al. 1998; Zhou et al. 2007), maize (Sanz-Alferez et al. 1995; Hulbert et al. 2001), wheat (Yahiaoui et al. 2004, 2009), and barley (Jorgensen 1994; Wei et al. 1999, 2002). The studies revealed that tandem and segmental gene duplication, recombination, diversifying selection, and sequence divergence in intergenic regions mainly contribute to the generation of novel *R* genes and their resistance specificity. This feature is coincident with gene evolution in flowering plants, which occurs preferentially at the ends of chromosomes and is derived by duplication and divergence associated with high rates of recombination (See et al. 2006). This feature explains why most of the *R* gene clusters are located in the distal regions of chromosomes.

Although many downy mildew resistance genes were identified from sunflower wild species [some of which were transferred into cultivated sunflower [(Miller and Gulya 1987, 1988, 1991; Seiler 1991; Rahim et al. 2002; Gulya 2005; Vear et al. 2008; Hulke et al. 2010; Qi et al. 2016)], none of these genes are present in confection sunflower. Pl_{19} is the first downy mildew *R* gene directly introduced from wild species into confection sunflower and is highly resistant to a wide range of *P. halstedii* races tested in the current study. However, deployment of a single gene in sunflower production will place strong selection pressure on the pathogen population, as it is known that *P. halstedii* pathogens quickly evolve and mutate to overcome plant resistances (Gascuel et al. 2015; Viranyi et al. 2015). To enhance the durability and effectiveness of a single resistance gene, Pl_{19} should be used in combination with other *Pl* genes. In an ongoing project, we selected three *Pl* genes, Pl_{Arg}, Pl_{17}, and Pl_{18}, and are transferring them from oil sunflower into confection sunflower to diversify the *Pl* gene pool in confection sunflower. Pl_{19} will easily combine with Pl_{Arg} and Pl_{18} in a single line, as the two genes are located in LGs 1 and 2, respectively (Wieckhorst et al. 2010; Qi et al. 2016). In contrast, the linkage between Pl_{17} and Pl_{19} appears to be tight in LG4, and a large segregating population from a cross of Pl_{17}/Pl_{19} is needed to recover rare recombinations. Because Pl_{Arg}, Pl_{17}, and Pl_{18} are resistant to all currently identified North America *P. halstedii* races (Gilley et al. 2016), the selection of genotypes with a gene combination by disease screening is not possible due to the lack of pathogen isolates with a specific virulence gene. Therefore, molecular markers for each gene are required for the selection of resistant pyramids with both parent's *Pl* genes from a segregation population. Such DNA markers are now available for Pl_{Arg}, Pl_{17}, and Pl_{18} (Wieckhorst et al. 2010; Qi et al. 2015, 2016); thus, the tightly linked SSR and SNP markers for Pl_{19} identified in this study will facilitate to pyramid this new gene with other *Pl* genes to aid in the development of durable downy mildew control in confection sunflower.

Author contribution statement Conceived and designed the experiments: LLQ. Performed the experiments: ZWZ, LLQ, GJM, JZ, SGM. Analyzed data: ZWZ, LLQ. Wrote the paper: LLQ, ZWZ. Commented on the manuscript before submission: SGM, GJM.

Acknowledgments The authors would like to thank Dr. Loren Rieseberg for providing access to the Sunflower Genome Data Repository. We also thank Drs. Zhao Liu and Steven Xu for critical review of the manuscript, Angelia Hogness for technical assistance, and Michelle Gilley for providing some downy mildew isolates of different races. ZWZ was supported by China Scholarship Council. This project was supported by the USDA-ARS CRIS Project No. 3060-21000-039-00D, and the USDA-AMS Specialty Crop Block Grant Program 14-SCBGP-ND-0038. Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. The USDA is an equal opportunity provider and employer.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments were performed in compliance with the current laws of the USA.

References

Albourie JM, Tourvieille J, Tourvieille De Labrouhe D (1998) Resistance to metalaxyl in isolates of the sunflower pathogen *Plasmopara halstedii*. European J Plant Pathol 104:2335–2342

Bachlava E, Radwan OE, Abratti G, Tang S, Gao W, Heesacker AF, Bazzalo ME, Zambelli A, Leon AJ, Knapp SJ (2011) Downy mildew (Pl_8 and Pl_{14}) and rust (R_{Adv}) resistance genes reside in close proximity to tandemly duplicated clusters of non-TIR-like NBS-LRR-encoding genes on sunflower chromosomes 1 and 13. Theor Appl Genet 122:1211–1221

Bouzidi MF, Badaoui S, Cambon F, Vear F, Tourvieille De Labrouhe D, Nicolas P, Mouzeyar S (2002) Molecular analysis of a major locus for resistance to downy mildew in sunflower with specific PCR-based markers. Theor Appl Genet 104:592–600

Bowers JE, Bachlava E, Brunick RL, Rieseberg LH, Knapp SJ et al (2012) Development of a 10,000 locus genetic map of the

sunflower genome based on multiple crosses. Genes Genomes Genetics 2:721–729

Ellis JG, Lawrence GJ, Finnegan EJ, Anderson PA (1995) Contrasting complexity of two rust resistance loci in flax. Proc Natl Acad Sci USA 92:4185–4188

Ellis JG, Lawrence GJ, Ayliffe M, Anderson P, Collins N, Finnegan J, Frost D, Luck J, Pryor T (1997) Advances in the molecular genetic analysis of the flax-flax rust interaction. Annu Rev Phytopathol 35:271–291

Ellis J, Dodds P, Pryor T (2000) Structure, function and evolution of plant disease resistance genes. Curr Opin Plant Biol 3:278–284

Fick GN, Zimmer DE (1974) RHA27, RHA273 and RHA274 sunflower parental lines for producing downy mildew resistant hybrids. Available: http://www.lib.ndsu.nodak.edu/repository/bitstream/handle/10365/9694/farm_32_02_02.pdf?sequence=1

Funk VA, Bayer RJ, Keeley S, Chan R, Watson L, Gemeinholzer B, Schilling E, Panero JL, Baldwin BG, Garcia-Jagas N, Susanna A, Jansen RK (2005) Everywhere but Antarctica: using a supertree to understand the diversity and distribution of the Compositae. Bol Skr 55:343–374

Gascuel Q, Martinez Y, Boniface MC, Vear F, Pichon M, Godiard L (2015) The sunflower downy mildew pathogen *Plasmopara halstedii*. Mol Plant Path 16:109–122

Gilley MA, Markell SG, Gulya TJ, Misar CG (2016) Prevalence and virulence of *Plasmopara halstedii* (downy mildew) in sunflowers. Available at http://www.sunflowernsa.com/uploads/research/1277/Prevalence.Downey_Gilley.etal_2016.rev.pdf

Gulya TJ (2005) Evaluation of wild annual *Helianthus* species for resistance to downy mildew and *Sclerotinia* stalk rot. In: Proceeding 27th sunflower research forum. Fargo ND, Jan 12–13, 2005 http://www.sunflowernsa.com/uploads/research/265/Gulya_WildHelianthus_studies_05.pdf

Gulya TJ, Draper M, Harbour J, Holen C, Knodel J, Lamey A, Mason P (1999) Metalaxyl resistance in sunflower downy mildew in North America. In: Proceedings of 21st sunflower research workshop. Fargo, ND, Jan 14–15, 1999. pp. 118–123

Gulya TJ, Markell S, McMullen M, Harveson B, Osborne L (2011) New virulent races of downy mildew: distribution, status of DM resistant hybrids, and USDA sources of resistance. In: Proceedings of 33th Sunflower Research Forum. Fargo, ND, Jan 12–13, 2011. https://www.sunflowernsa.com/uploads/resources/575/gulya_virulentracesdownymildew.pdf

Gulya T, Kandel H, McMullen M, Berglund D, Mathew F, Lamey H, Nowatzki J, Markell S (2013) Prevalence and incidence of sunflower downy mildew in North Dakota between 2001 and 2011. Plant Health Progress. doi:10.1094/PHP2013-022-01-RS

Harveson R, Markell S, Block C, Gulya T (2016) Compendium of sunflower diseases. American Phytopathology Press, St Paul. p. 40

Hladni N (2016) Present status and future prospects of global confectionary sunflower production. In: Proceedings of 19th international sunflower conference. Edirne, Turkey, May 29–June 3, 2016. pp. 47–60

Hulbert SH, Webb CA, Smith SM, Sun Q (2001) Resistance gene complexes: evolution and utilization. Ann Rev Phytopathol 39:285–312

Hulke BS, Miller JF, Gulya TJ, Vick BA (2010) Registration of the oilseed sunflower genetic stocks HA 458, HA 459, and HA 460 possessing genes for resistance to downy mildew. J Plant Reg 4:93–97

Hulke BS, Grassa CJ, Bowers JE, Burke JM, Qi LL, Talukder ZI, Rieseberg LH (2015) A unified single nucleotide polymorphism map of sunflower (*Helianthus annuus* L.) derived from current genomic resources. Crop Sci 55:1696–1702

Jones DA, Dickinson MJ, Balint-Kurti PJ, Dixon MS, Jones JDC (1993) Two complex resistance loci revealed on tomato by classical and RFLP mapping of *Cf*-2, *Cf*-4, *Cf*-5, and *Cf*-9 genes for resistance to *Cladosporium fulvum*. Mol Plant-Microbe Interac 6:348–357

Jorgensen JH (1994) Genetics of powdery mildew resistance in barley. Crit Rev Plant Sci 13:97–119

Kaloshian I, Lange WH, Williamson VM (1995) An aphid-resistance locus is tightly linked to the nematode-resistance gene *Mi* in tomato. Proc Natl Acad Sci 92:622–625

Kantar MB, Sosa CC, Khoury CK, Castañeda-Álvarez NP, Achicanoy HA, Bernau V, Kane NC, Marek L, Seiler G, Rieseberg LH (2015) Ecogeography and utility to plant breeding of the crop wild relatives of sunflower (*Helianthus annuus* L). Frontiers in Plant Science 6:841

Kesseli RV, Paran I, Michelmore RW (1994) Analysis of a detailed genetic linkage map of *Lactuca sativa* (lettuce) constructed from RFLP and RAPD markers. Genetics 136:1435–1446

Kunkel BN (1996) A useful weed put to work-genetic analysis of disease resistance in *Arabidopsis thaliana*. Trends Gene 12:63–69

Leister D (2004) Tandem and segmental gene duplication and recombination in the evolution of plant disease resistance genes. Trends Genet 20:116–122

Liu Z, Gulya TJ, Seiler GJ, Vick BA, Jan CC (2012) Molecular mapping of the Pl_{16} downy mildew resistance gene from HA-R4 to facilitate marker-assisted selection in sunflower. Theor Appl Genet 125:121–131

Markell SG, Harveson RM, Block CC, Gulya TJ (2015) Sunflower diseases. In: Martínez-Force E, Dunford NT, Salas JJ (eds) Sunflower: chemistry, production, processing and utilization. AOCS Press, Urbana, p 730

Michelmore RW, Meyers BC (1998) Cluster of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res 8:1113–1130

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Miller JF, Gulya TJ (1987) Inheritance of resistance to race three downy mildew in sunflower. Crop Sci 27:210–212

Miller JF, Gulya TJ (1988) Registration of six downy mildew resistant sunflower germplasm lines. Crop Sci 28:1040–1041

Miller JF, Gulya TJ (1991) Inheritance of resistance to race four of downy mildew derived from interspecific crosses in sunflower. Crop Sci 31:40–43

Mulpuri S, Liu Z, Feng J, Gulya TJ, Jan CC (2009) Inheritance and molecular mapping of a downy mildew resistance gene, Pl_{13} in cultivated sunflower (*Helianthus* annuus L.). Theor Appl Genet 119:795–803

National Sunflower Association (2011) Confection sunflower update. http://www.sunflowernsa.com/magazine/articles/default.aspx?ArticleID=3362

Nishimura M (1922) Study in *Plasmopara halstedii*. J Coll Agric Hokkaido Imp Univ XI 3:185–210

Novotelnova NS (1960) Biological characteristics of *Plasmopara halstedii* (Farl.) Berl. Et de Toni on sunflower. Botan Zhur 45:1283–1300 **(In Russian)**

Qi LL, Seiler GJ (2016) Registration of an oilseed sunflower germplasm HA-DM1 resistant to sunflower downy mildew. Journal of Plant Registrations. 10:195–199

Qi LL, Gulya TJ, Seiler GJ, Hulke BS, Vick BA (2011) Identification of resistance to new virulent races of rust in sunflowers and validation of DNA markers in the gene pool. Phytopathol 101:241–249

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

Rahim M, Jan CC, Gulya TJ (2002) Inheritance of resistance to sunflower downy mildew races 1, 2 and 3 in cultivated sunflower. Plant Breed 121:57–60

Rogers CE, Thompson TE, Seiler GJ (1982) Sunflower species of the United States. National Sunflower Association, Bismarck, pp 4–22

Sanz-Alferez S, Richter TE, Hulbert SH, Bennetzen JL (1995) The *Rp3* disease resistance locus of maize: mapping and characterization of introgressed alleles. Theor Appl Genet 91:25–32

Schilling EE (2006) Helianthus. Flora of North America Committee. Oxford University Press, New York, pp 141–169

See DR, Brooks SA, Nelson JC, Brown-Guedira GL, Friebe B, Gill BS (2006) Gene evolution at the ends of wheat chromosomes. Proc Natl Acad Sci USA 103:4162–4167

Seiler GJ (1991) Registration of 13 downy mildew tolerant interspecific sunflower germplasm lines derived from wild annual species. Crop Sci 31:1714–1716

Seiler GJ (2010) Utilization of wild *Heliathus* species in breed for disease resistance. In: Proceedings of the international symposium "sunflower breeding on resistance to diseases", Krasnodar, Russia. International Sunflower Association, Paris, France. June 23–24, 2010. pp 37–51

Seiler GJ, Jan CC (2010) Basic information. In: Hu J, Seiler G (eds) Genetics, genomics and breeding of sunflower. Science Publishers, Enfield, pp 1–50

Seiler GJ, Marek LF (2014) USDA-ARS sunflower germplasm collections. http://www.sunflowernsa.com/uploads/20/usda.ars.germplasm_seiler_2014.pdf

Slabaugh MB, Yu JK, Tang SX, Heesacker A, Hu X, Lu GH, Bidney D, Han F, Knapp SJ (2003) Haplotyping and mapping a large cluster of downy mildew resistance gene candidates in sunflower using multilocus intron fragment length polymorphisms. Plant Biotechnol J 1:167–185

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauguet C, Ronald PC (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270:1804–1806

Song WY, Pi LY, Wang GL, Gardner J, Holsten T, Ronald PC (1997) Evolution of the rice *Xa21* disease resistance gene family. Plant Cell 9:1279–1287

Stebbins JC, Winchell CJ, Constable JVH (2013) *Helianthus winteri* (Asteraceae), a new perennial species from the southern Sierra Nevad a foothills, California. Aliso 31:19–24. doi:10.5642/aliso.20133101.04

Takken FL, Tomas CM, Joosten MH, Golstein C, Westerink N, Hille J, Nijkamp HJ, De Wit PJ, Jones JDJ (1999) A second gene at the tomato *Cf*-4 locus confers resistance to *Cladosporium fulvum* through recognition of a novel avirulence determinant. Plant J 20:279–288

Talukder ZI, Gong L, Hulke BS, Pegadaraju V, Song QJ, Schultz Q, Qi LL (2014) A high-density SNP map of sunflower derived from RAD-sequencing facilitating fine-mapping of the rust resistance gene R_{12}. PLoS ONE 9(7):e98628. doi:10.1371/journal.pone.0098628

Tang S, Yu JK, Slabaugh MB, Shintani DK, Knapp SJ (2002) Simple sequence repeat map of the sunflower genome. Theor Appl Genet 105:1124–1136

Van Ooijen JW (2006) JoinMap ® 4—Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen

Vear F, Seriveys H, Petit A, Serre F, Boudon JP, Roche S, Walser P, Tourvieille De Labrouhe D (2008) Origins of major genes for downy mildew resistance in sunflower. In: Proceedings of 17th international sunflower conference cordoba, Spain. Consejeria de Agricultura y Pesca. pp 125–130

Viranyi F, Gulya TJ, Tourieille DL (2015) Recent changes in the pathogenic variability of *Plasmopara halstedii* (sunflower downy mildew) populations from different continents. Helia. doi:10.1515/helia-2015-000938:149-162

Wang GL, Ruan DL, Song WY, Sideris S, Chen LL, Pi LY, Zhang S, Zhang Z, Fauquet C, Gaut BS, Whalen MC, Ronald PC (1998) *Xa21D* encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. Plant Cell 10:765–779

Wei F, Gobelman-Werner K, Morrol SM, Kurth J, Mao L, Wing R, Leister D, Schulze-Lefert P, Wise RP (1999) The *Mla* (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153:1929–1948

Wei F, Wong RA, Wise RP (2002) Genome dynamics and evolution of the *Mla* (powdery mildew) resistance locus in barley. Plant Cell 14:1903–1917

Wieckhorst S, Bachlava E, Dußle CM, Tang S, Gao W, Saski C, Knapp SJ, Schön CC, Hahn V, Bauer E (2010) Fine mapping of the sunflower resistance locus Pl_{ARG} introduced from the wild species *Helianthus argophyllus*. Theor Appl Genet 121:1633–1644

Yahiaoui N, Srichumpa P, Duadler R, Keller B (2004) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. Plant J 37:528–538

Yahiaoui N, Kaur N, Keller B (2009) Independent evolution of functional *Pm3* resistance gens in wild tetraploid wheat and domesticated bread wheat. Plant J 57:846–856

Young PA, Morris HE (1927) Plasmopara downy mildew of cultivated sunflower. Am J Bot 14:551–558

Yu JK, Tang S, Slabaugh MB, Heesacker A, Cole G et al (2003) Towards a saturated molecular genetic linkage map for cultivated sunflower. Crop Sci 43:367–387

Zhou B, Dolan M, Sakai H, Wang WL (2007) The genomic dynamics and evolutionary mechanism of *Pi2/9* locus in rice. Mol Plant-Microbe Interac 20:63–71

Zimmer DE (1974) Physiological specialization between races of *Plasmopara halstedii* in America and Europe. Phytopathol 64:1465–1467