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ORIGINAL PAPER

Pl$_{17}$ is a novel gene independent of known downy mildew resistance genes in the cultivated sunflower (*Helianthus annuus* L.)

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Abstract

Key message ***Pl*$_{17}$, a novel downy mildew resistance gene independent of known downy mildew resistance genes in sunflowers, was genetically mapped to linkage group 4 of the sunflower genome.**

Abstract Downy mildew (DM), caused by *Plasmopara halstedii* (Farl.). Berl. et de Toni, is one of the serious sunflower diseases in the world due to its high virulence and the variability of the pathogen. DM resistance in the USDA inbred line, HA 458, has been shown to be effective against all virulent races of *P. halstedii* currently identified in the USA. To determine the chromosomal location of this resistance, 186 $F_{2:3}$ families derived from a cross of HA 458 with HA 234 were phenotyped for their resistance to race 734 of *P. halstedii*. The segregation ratio of the population supported that the resistance was controlled by a single dominant gene, *Pl*$_{17}$. Simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) primers were used to identify molecular markers linked to *Pl*$_{17}$. Bulked segregant analysis using 849 SSR markers located *Pl*$_{17}$ to linkage group (LG) 4, which is the first DM gene discovered in this linkage group. An F_2 population of 186 individuals was screened with polymorphic SSR and SNP primers from LG4. Two flanking markers, SNP SFW04052 and SSR ORS963, delineated *Pl*$_{17}$ in an interval of 3.0 cM. The markers linked to *Pl*$_{17}$ were validated in a BC_3 population. A search for the physical location of flanking markers in sunflower genome sequences revealed that the *Pl*$_{17}$ region had a recombination frequency of 0.59 Mb/cM, which was a fourfold higher recombination rate relative to the genomic average. This region can be considered amenable to molecular manipulation for further map-based cloning of *Pl*$_{17}$.

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Introduction

Sunflower downy mildew (DM), caused by *Plasmopara halstedii* (Farl.). Berl. et de Toni, is a widespread disease with regular occurrence in the USA and worldwide. The disease can cause heavy yield losses of 50–95 % in cool, wet years and adversely affects other aspects of seed quality (Molinero-Ruiz et al. 2003). Because of this destructive potential, DM is one of the most serious sunflower diseases. Current fungicides for seed treatment are only partially effective as preventative measures and there are no foliar curative fungicides. The use of resistant hybrids, where available, is the most efficient measure of controlling downy mildew.

Genetic studies of resistance to downy mildew in sunflowers have indicated that the sources of resistance are controlled by single genes denoted *Pl*, which are usually dominant (Zimmer and Kinman 1972; Miller and Gulya 1987, 1991; Tan et al. 1992; Molinero-Ruiz et al. 2003).

The most common breeding strategy for resistance to downy mildew in sunflowers has been the use of race-specific resistance, which follows the gene-for-gene hypothesis (Flor 1955). The deployment of individual resistance genes often results in the loss of resistance in a relatively short period due to the co-evolution of host and pathogen, resulting in the emergence of new races of the pathogen that possess the corresponding virulence genes. Thus, the discovery of new sources of resistance and the use of broad-spectrum resistance by combining effective and diverse *Pl* genes (gene pyramiding) are the best strategies for avoiding fast breakdown of resistance.

Currently, 18 downy mildew resistance genes (Pl_1–Pl_{16}, Pl_{21}, and Pl_{ARG}) have been reported in cultivated and wild species of sunflowers (Zimmer and Kinman 1972; Miller and Gulya 1991; Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997, 2008; Rahim et al. 2002; Molinero-Ruiz et al. 2003; Vear et al. 2008; Mulpuri et al. 2009; de Romano et al. 2010; Bachlava et al. 2011; Liu et al. 2012). However, many of these genes no longer condition resistance to most races, because virulent downy mildew races have increased in prevalence in response to the release of sunflower hybrids with race-specific resistance genes (Roeckel-Drevet et al. 1997, 2003; Molinero-Ruiz et al. 2000; Rashid et al. 2006; Gulya 2007; Gulya et al. 2011). The USDA-released inbred lines HA 335 and HA 336 (which carry Pl_6), HA 337, HA 338, and HA 339 (which all possess Pl_7), and RHA 340 (which harbors Pl_8) have been extensively used to produce DM-resistant hybrids worldwide (Miller and Gulya 1988, 1991). However, the emergence of virulent downy mildew races has rendered most of the commercial hybrids susceptible to new virulent races. At least 36 physiological races of *P. halstedii* have been identified around the world, with North America (24 races) and Europe (21 races) containing the highest number of races (Roeckel-Drevet et al. 1997, 2003; Tourvieille de Labrouhe et al. 2000, 2005; Tosi and Zazzerini 2004; Tosi and Beccari 2007; Gulya 2007; Gulya et al. 2011). As of 2010, five North American (NA) races, 314, 704, 714, 734, and 774, and at least eight new races in France have been identified as virulent against Pl_6 and Pl_7 (Gulya et al. 2011). The identification of races conferring virulence against two of the most utilized resistance genes in commercial sunflowers poses a serious threat to sunflower production in the world and has spurred the search for new sources of resistance.

The downy mildew resistance in the USDA inbred line HA 458 has been shown to be effective against all new virulent downy mildew races in the USA and has not been used in sunflower hybrid production (Hulke et al. 2010; Gulya et al. 2011). The source of resistance for HA 458 is derived from wild collections of *H. annuus* from Idaho, USA (PI 468435). Incorporating several of the new genes into sunflower commercial hybrids should mitigate the threat posed by current virulent downy mildew races. This can be done most efficiently only once the identity and position of the new *Pl* genes are determined. Molecular mapping of resistance derived from HA 458 is necessary to conclude whether its resistance gene is different from known *Pl* genes. Markers closely linked to this presumed new gene will provide a useful tool for speeding up the deployment of the gene in commercial sunflower hybrid production.

Materials and methods

Plant materials

The mapping populations of F_2 and F_2-derived F_3 generations were developed from the cross of the DM-resistant parent HA 458 with the DM-susceptible parent HA 234. HA 458 (PI 655009) is a BC_2F_6-derived BC_2F_7 maintainer genetic stock selected from the cross HA 434*3/PI 468435. HA 434 (PI 633744) is a high oleic fatty acid germplasm line, and PI 468435 is a wild *Helianthus annuus* L. accession collected in 1979 near Caldwell, Idaho, and serves as a donor of downy mildew resistance. HA 458 was released by the USDA-ARS and the North Dakota Agricultural Experiment Station in 2010 as a high oleic fatty acid germplasm line combined with downy mildew resistance (Hulke et al. 2010). HA 234 is a sunflower maintainer line susceptible to downy mildew and was released by the USDA-ARS and the North Dakota Agricultural Experiment Station in 1971. A total of 225 F_2 plants were grown in the greenhouse in 2011 and advanced to the F_3 generation.

A BC_3 population derived from HA-R6*4/HA 458 was used to validate the Pl_{17} specific markers. HA-R6 (PI 607509) is a confection maintainer line released by the USDA and the North Dakota Agricultural Experiment Station in 2001 as a rust-resistant line. HA 458 was used as a downy mildew-resistant donor to transfer the gene conferring DM resistance into confection sunflowers.

Downy mildew inoculation and phenotyping

The whole seedling immersion method described by Gulya et al. (1991) was applied for seedling tests using the North America (NA) downy mildew race 734, which is a new, virulent race identified in the USA in 2010 (Gulya et al. 2011). Briefly, seeds were surface sterilized with 20 % bleach solution (~1 % sodium hypochlorite) for 10 min, rinsed well with tap water, evenly spaced on germination paper, and incubated in a germinator in the dark at 22–24 °C for 3 days. Freshly produced spores of race 734 were used to inoculate 3-day-old seedlings by immersion for 3–5 h. in

suspensions of 2–4 × 10^4 zoosporangia at 18 °C. Inoculated seedlings were grown in a pasteurized mixture of sand and perlite (2:3, v/v) in the greenhouse (24 ± 3 °C, 16-h photoperiod) for 12–14 days. The plants were placed in a chamber maintained at 18 °C and 100 % relative humidity overnight to induce sporulation and were then returned to the greenhouse. A plant was considered susceptible (S) if sporulation was observed on cotyledons and true leaves, and resistant (R) if no sporulation was observed.

A total of 25–30 seeds from each of 186 F_3 families along with the two parental lines, HA 234 and HA 458, were inoculated with NA race 734 and tested for their downy mildew resistance in the greenhouse in June 2012. The F_3 families were classified as homozygous resistant if none of the seedlings had sporulation, segregating if some seedlings (about one-quarter in a F_3 family) had sporulation on the cotyledons and true leaves, and homozygous susceptible if all seedlings had sporulation on cotyledons and true leaves.

DNA extraction and PCR conditions

Genomic DNA was isolated from the young leaves of the parents and F_2 individuals using the 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).

For simple sequence repeat (SSR) markers, polymerase chain reaction (PCR) was conducted on a Peltier thermocycler (Bio-Rad Lab, Hercules, CA, USA) with a touchdown program as described by Qi et al. (2011). The PCR products were diluted 20- to 120-fold before analysis depending on the yield of the PCR products. The PCR-amplified fragments were separated according to size with an IR2 4200/4300 DNA Analyzer (Li-COR, Lincoln, NE, USA). All fragment sizes included 19 bp of the M13 fluorescent tag.

Bulked segregant analysis (BSA) and linkage mapping

Initially, a total of 849 simple sequence repeat (SSR) markers were used to screen two parents, HA 458 and HA 234. Subsequently, bulked segregant analysis was performed using the polymorphic SSR markers. DNA from the F_2 population was bulked into two groups: the R-bulk consisted of ten homozygous resistant F_2 plants, and the S-bulk consisted of ten homozygous susceptible F_2 plants based on the phenotypic response of the F_3 generation to downy mildew race 734. The markers that showed a polymorphic pattern between the R and S bulks were considered to be potentially linked to the resistance gene and were further evaluated in the F_2 population. JoinMap 4.1 was used for linkage analyses and map construction with a regression mapping algorithm and Kosambi's mapping function (Van Ooijen 2006). The Chi-square (χ^2) test was used to assess goodness of fit to the expected segregation ratio for each marker. A minimum likelihood of odds (LOD) $\geq$3.0 and a maximum distance of $\leq$50 centimorgans (cM) were used to test linkage among markers.

Map saturation and SNP genotyping

A total of 41 single nucleotide polymorphism (SNP) markers that possibly covered the *Pl* gene region in LG4 were selected by comparison of the map positions of common SSR markers to the published SNP maps (Bowers et al. 2012; Talukder et al. 2014). Out of 41 SNPs, 16 were selected from the SNP map developed by the National Sunflower SNP Consortium (hereafter referred to as NSA SNPs, Table S1, Talukder et al. 2014) and 25 were selected from the Bower's SNP map (hereafter referred to as SFW SNPs, Table S2, Bowers et al. 2012). The 16 NSA SNPs and 25 SFW SNPs covered a region of 13.86 and 15.62 cM on LG4, respectively (Tables S1, S2).

Genotyping of the parental lines and F_2 population with NSA SNPs was conducted by BioDiagnostics Inc. (River Falls, WI, USA), where the NSA SNPs were developed (Pegadaraju et al. 2013). Genotyping of the SFW SNPs was performed using a strategy of converting the SNPs into length polymorphism markers. Briefly, for each SNP, two-tailed forward allele-specific primers (AS-primers F1 and F2) and one common reverse primer were designed. An additional 5-base oligonucleotide (5′-ATGAC-3′) was inserted between the tail and the allele-specific sequences in the AS-primer F2 to produce a length difference between the two alleles after amplification (Table 1). A universal priming-element-adjustable primer (PEA-primer 5-ATAGCTGG-Sp9-GCAACAGGAACCAGCTATGAC-3) with an attached fluorescence tag at the 5′ terminus was used in each PCR reaction. The conditions of the SNP PCR reactions have been described by Qi et al. (2015).

Results

Inheritance of downy mildew resistance

The F_3 progenies derived from F_2 plants and the two parents, HA 234 and HA 458, were inoculated with *P. halstedii* race 734. As expected, HA 234 was highly susceptible to race 734 and showed sporulation on cotyledons and true leaves, whereas HA 458 was resistant to race 734 and showed no sporulation on cotyledons. Resistance reactions similar to those of HA 458 were observed in the F_3 population. Of the 186 F_3 families, 50 were homozygous resistant,

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
SFW08268	SFW08268F1a	GCAACAGGAACCAGCTATGACAAGTTACACCGGAACATCTT
	SFW08268F2a	GCAACAGGAACCAGCTATGAC*ATGAC*AAGTTACACCGGAACAGTTC
	SFW08268R	GATGTTGGTGAATGGTGGGTAT
NSA_003564	NSA_003564F1	GCAACAGGAACCAGCTATGACTTTCAGCTACCGTACAGCAAAAA
	NSA_003564F2	GCAACAGGAACCAGCTATGAC*ATGAC*TTTCAGCTACCGTACAGCAGGAG
	NSA_003564R	ATGTCACATGCACCACAAAT

The tail sequence is underlined and the additional five-base oligonucleotide insertion in AS-primer F_2 is given in italic

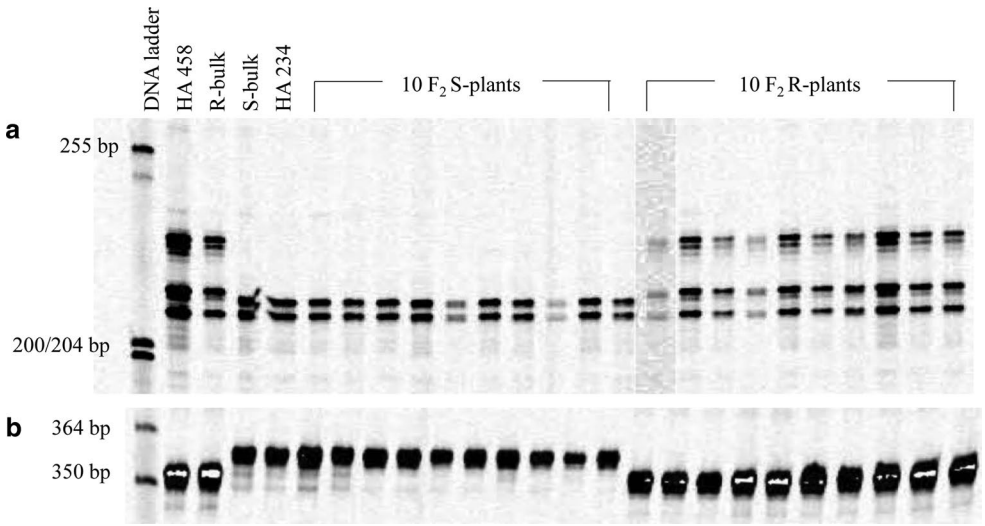

Fig. 1 Bulked segregant analysis of SSR markers ORS1197 and ORS963; PCR pattern of ORS1197 (**a**) and ORS963 (**b**). The ten homozygous susceptible F_2 plants came from the S-bulk, and the ten homozygous resistant F_2 plants came from the R-bulk

89 were heterozygous, and 47 were homozygous susceptible. The segregation of downy mildew resistance for 186 $F_{2:3}$ families fit a 1:2:1 segregation ratio ($\chi^2 = 0.441$, $P = 0.802$), indicating that resistance from HA 458 is controlled by a single dominant gene as previously proposed (Hulke et al. 2010). This gene was designated Pl_{17}.

Bulked segregant analysis (BSA) and linkage mapping

The two parents, HA 234 and HA 458, were screened with 849 SSR markers that provided coverage of the sunflower genome. Out of these, 361 markers identified polymorphic DNA fragments in the parents, covering a total of 17 linkage groups (LGs) with the number ranging from 8 for LG12 and 35 for LG16. The bulk segregant analysis (Michelmore et al. 1991) was conducted using polymorphic SSR markers in the parents and the S- and R-bulks. ORS1197 and ORS963 SSR primers located on LG4 of the sunflower genome generated polymorphic DNA fragments between the two bulks. All homozygous susceptible plants from the S-bulk showed the HA 234 allele, whereas ten homozygous resistant plants from the R-bulk gave the HA 458 allele (Fig. 1).

To confirm linkage with Pl_{17}, ORS1197, ORS963, and 14 additional polymorphic SSR primers from LG4 were used to amplify DNA from the 186 F_2 individuals. The 12 SSR markers (3 dominant, 9 co-dominant) were placed on the linkage map, covering a genetic distance of 26.3 cM with a gap of 9.4 cM between markers ORS963 and HT664. The co-dominant SSR markers, ORS1197 and ORS963, flanked Pl_{17} at a genetic distance of 2.8 and 0.9 cM, respectively (Fig. 2b).

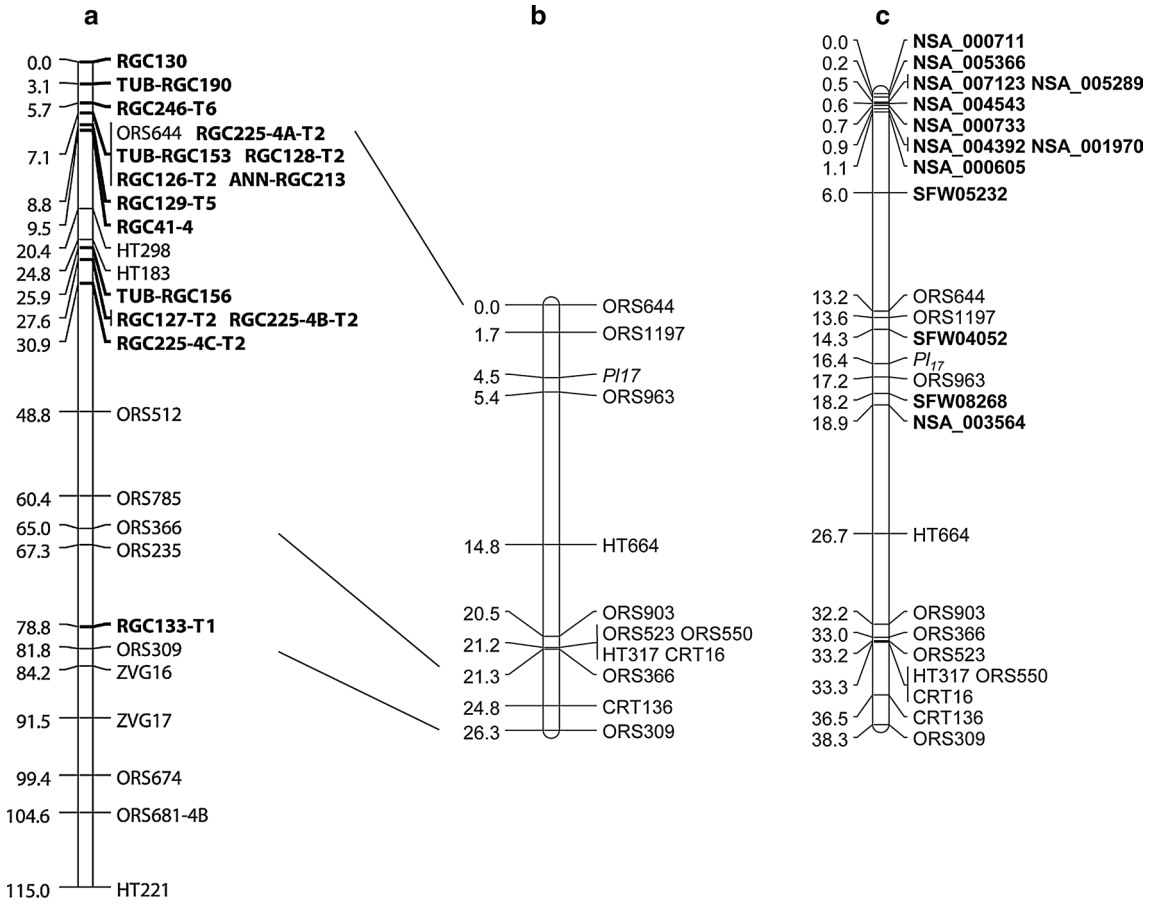

Fig. 2 Genetic maps of sunflower linkage group (LG) 4. **a** LG4 genetic map of resistance gene candidates (RGCs) taken from Radwan et al. (2008), showing that Pl_{17} was mapped close to an RGC cluster in LG4, **b** LG4 SSR map of Pl_{17}, **c** LG4 SSR and SNP combined map of Pl_{17}. SNP markers are in *boldface*

Map saturation

To further saturate the region where Pl_{17} resides, we selected 41 SNP markers from the two SNP genetic maps based on the position of common SSR markers (Bowers et al. 2012; Talukder et al. 2014). Sixteen SNPs were selected from the NSA SNP map, covering a region of 0.00–13.86 cM with a gap of 10.19 cM between NSA_004244 and NSA_000781 (Table S1, Talukder et al. 2014). Out of these, 12 SNPs gave allele differences between the parents, 11 of which were concentrated to the 0–2.46 cM region. By contrast, only one polymorphic SNP, NSA_003564, was located in the 13.86 cM region (Table S1). These polymorphic SNPs were subsequently genotyped in the F_2 population. Data for three SNPs, NSA_003995, NSA_001186, and NSA_003564, were excluded from further analysis due to insufficient data, leaving a total of nine SNPs for the final linkage analysis. In the genetic map, these nine SNP markers represented a coherent linkage block at the top of LG4 and covered a genetic distance of 4.0 cM. This map position was consistent with their position in the original SNP map (Table 2), and none of these SNPs were found to be closely linked to Pl_{17} (Fig. 2c).

NSA_003564 is a marker that may be close to Pl_{17} based on its SNP map position, but because it failed to map with the available genotyping data, we converted this SNP into a length polymorphism marker. Three primers were designed (Table 1) and used to amplify the F_2 population and the two parents. The primers amplified two fragments in HA 234 and one fragment in HA 458. The absence of a bottom band in HA 458 indicated that this was a dominant marker (Fig. 3a). Linkage analysis mapped NSA_003564 to 2.5 cM proximal to Pl_{17} (Fig. 2c).

Out of the 25 SFW SNPs selected, three were polymorphic between HA 234 and HA 458. Recombination mapping showed that three SNPs were placed at a genetic interval of 12.2 cM with an order in agreement with that of their original SNP map (Fig. 2c, Table 2). Two SNPs, SFW04052 and SFW08268, flanked Pl_{17} at a genetic distance of 2.1 and 1.7 cM, respectively. In the combined SSR and SNP map of LG4, SFW04052 and ORS963 were the

Table 2 Blast search results of SNP sequences aligned to sunflower genome sequences and their physical positions in the sunflower genome

cM*	cM**	Query id	Subject id	% Identity	Alignment length	Mismatches	Gap opens	Q. start	Q. end	S. start	S. end	E-value	Bit score	Physical position
0.00	0	NSA_000711	scf7180037972694-2	99.75	393	0	1	1	393	6266	5875	0	719	LG_04: 0–33877 (33,878 bp)
0.00	0.2	NSA_005366	scf7180037972694-2	99.79	477	0	1	1	477	5234	5709	0	874	LG_04: 0–33877 (33,878 bp)
1.25	0.5	NSA_005289	scf7180038209529-0	99.55	222	0	1	1	222	783	1003	1.00E–110	403	LG_04: 254367–275330 (20,964 bp)
1.25	0.5	NSA_007123	scf7180038209529-0	99.61	259	0	1	1	259	993	736	4.00E–131	472	LG_04: 254367–275330 (20,964 bp)
1.25	1.1	NSA_000605	scf7180038311093-1	99.76	417	0	1	1	417	1795	1380	0	763	LG_04: 472887–505960 (33,074 bp)
1.25	0.7	NSA_000733	scf7180038311093-1	98.97	389	0	2	1	389	789	1173	0	693	LG_04: 472887–505960 (33,074 bp)
1.25	0.9	NSA_001970	scf7180038316969-6	96.54	376	5	2	1	376	796	1163	3.00E–174	616	LG_04: 505960–556395 (50,436 bp)
1.25	0.9	NSA_004392	scf7180038316969-6	98.45	322	4	1	1	322	938	618	2.00E–159	566	LG_04: 505960–556395 (50,436 bp)
1.25	0.6	NSA_004543	scf7180038316969-3	98.53	407	4	2	1	407	16456	16860	0	717	LG_04: 505960–556395 (50,436 bp)
5.21	6.0	SFW05232	scf7180038270171-2	98.35	121	1	1	1	121	1220	1339	1.00E–53	213	LG_04: 1384035–1401943 (17,909 bp)
12.10	14.3	SFW04052	scf7180038077014-6	98.92	93	0	1	1	93	6587	6678	2.00E–39	165	LG_04: 2224767–2270609 (45,843 bp)
	16.4	Pl_{17}												
13.86	18.9	NSA_003564	scf7180037925097	99.66	290	0	1	1	290	10590	10878	3.00E–148	529	LG_04: 3895530–3922086 (26,557 bp)
15.62	18.2	SFW08268	scf7180038321775-12	97.96	98	1	1	24	121	7630	7534	2.00E–40	169	LG_04: 4379426–4476308 (96,883 bp)

* Taken from Bowers et al. (2012) for SFW SNPs and Talukder et al. (2014) for NSA SNPs. ** Current study

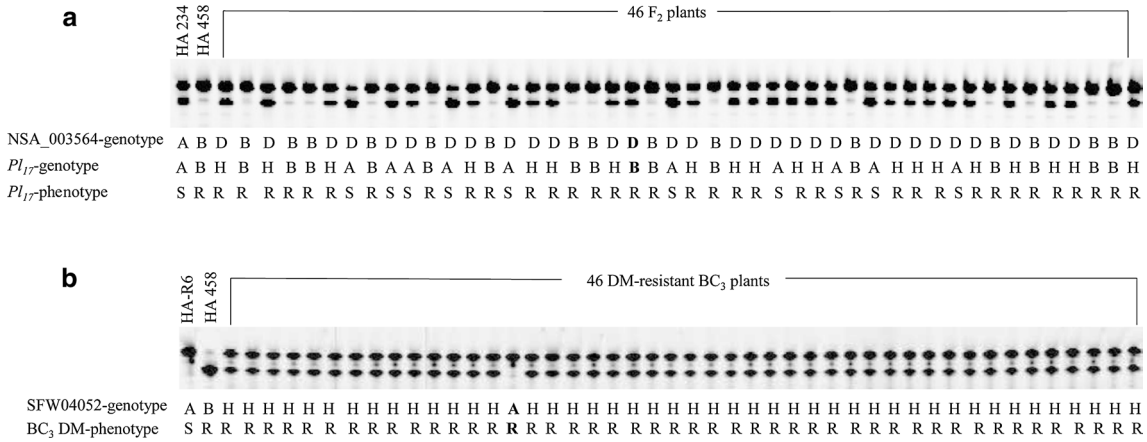

Fig. 3 PCR amplifications of the primers NSA_003564 (**a**) and SFW04052 (**b**). **a** PCR pattern of NSA_003564 in an F_2 segregation population of HA 234 × HA 458 (showing 46 of 186 F_2 plants from lanes 3 to 48). The PCR pattern of NSA_003564 primers for the heterozygous F_2 plants is the same as that of HA 234. F_2 genotypes for the Pl_{17} gene were determined by an $F_{2:3}$ progeny test. The symbol *A* represents homozygous HA 234 (genotype AA); *B* homozygous HA 458 (genotype BB); *H* heterozygous (genotype AB); *D* either homozygous HA 234 (AA) or heterozygous (AB). *S* susceptible, *R* resistant. **b** PCR pattern of SFW04052 in selected resistant BC_3 plants of HA-R6 × HA 458. The *bold capital letters* in (**a**) and (**b**) indicate sites of recombination

two closest flanking markers linked to Pl_{17} at a genetic distance of 2.1 and 0.8 cM, respectively (Fig. 2c).

Physical positions of SNP markers in the sunflower genome

The sequences of 13 mapped SNPs were blasted against the sunflower whole genome sequence of HA412 v0.2 assembly available at http://sunflowergenome.org/early_access/repository/main/genome_browser/blast.html.

These SNPs covered 18.9 cM in the current genetic map and aligned to eight scaffolds located between 0 and 4,476,308 bp in LG4. The recombination frequency in this interval was 0.24 Mb/cM (Table 2). The nine NSA SNPs clustered at the top of the LG4 genetic map and aligned to four scaffolds, which covered a physical length of 556,395 bp. By contrast, the one NSA SNP and three SFW SNPs aligned to four scaffolds and were located between 1,384,035 and 4,476,308 bp, covering a physical length of 3,092,273 bp. The physical length between SFW04052 and SFW 08268, the two SFW SNPs flanking Pl_{17}, was 2,251,471 bp and had a recombination rate of 0.59 Mb/cM (Table 2).

Marker validation in a BC_3 population

A total of 231 BC_3 plants derived from the backcross of HA-R6*4/HA 458 and the two parents HA-R6 and HA 458 were first inoculated with NA race 734. HA-R6 and 146 BC_3 plants were susceptible, while HA 458 and 85 BC_3 plants were resistant. Subsequently, the BC_3-resistant plants were genotyped using two SNP markers, SFW04052 and SFW08268, which flanked Pl_{17}. All but two of the plants were heterozygous for the two markers and presented both the HA 234 and HA 458 alleles, as expected. This result confirmed that these markers were associated with DM resistance (Table S3, Fig. 3b). Two recombination events were detected in plants 14-116-15 and 14-117-7, with one occurring between SFW04052 and Pl_{17} in 14-116-15 and the other occurring between Pl_{17} and SFW08268 in 14-117-7. Plant 14-116-15 had the HA-R6 allele of SFW04052 only but was DM resistant, whereas 14-117-7 was DM resistant but had only the HA-R6 allele of SFW08268 (Table S3, Fig. 3b). The recombination rate was similar to that detected in the F_2 population.

Discussion

The recently released inbred line HA 458 is a highly valuable source of resistance to downy mildew (Hulke et al. 2010). The Pl_{17} identified in this line is a broad-spectrum gene that confers resistance to all races of *P. halstedii* currently identified in North America (Gulya et al. 2011). Out of 18 *Pl* resistance genes (Pl_1–Pl_{16}, Pl_{21}, and Pl_{ARG}) previously reported in sunflowers, 12 have been placed on the sunflower genetic map and cluster into three linkage groups that correspond to LGs 1, 8, and 13 of the public sunflower SSR map (Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997; Rahim et al. 2002, Molinero-Ruiz et al. 2003; Yu et al. 2003; Mulpuri et al. 2009; de Romano et al. 2010; Bachlava et al. 2011; Liu et al. 2012). Pl_{17}, which was mapped in the present study, is the first *Pl* gene located on LG4, indicating that this is a novel gene

independent of the known *Pl* gene clusters that can be easily pyramided with other *Pl* genes from different linkage groups to enhance the durability of field resistance.

Many resistance genes in plants are known to be organized into gene clusters as is the case of sunflower *Pl* genes (Islam and Shepherd 1991; Jones et al. 1993; Song et al. 1997; Meyers et al. 1998; Michelmore and Meyers 1998; Richter and Ronald 2000; Hulbert et al. 2001; Wei et al. 2002; Huang et al. 2003). Among the 12 previously mapped *Pl* genes, 4 are in LG1 (Pl_{13}, Pl_{14}, Pl_{16}, and Pl_{ARG}), 5 in LG8 (Pl_1, Pl_2, Pl_6, Pl_7, and Pl_{15}), and 3 in LG13 (Pl_5, Pl_8, Pl_{21}). The *Pl* gene clusters in LGs 8 and 13 correspond, respectively, to the first and second largest nucleotide-binding site and leucine-rich repeats (NBS-LRR) clusters identified in these two linkage groups in sunflowers (Radwan et al. 2008). Three genes in LG1, Pl_{13}, Pl_{14}, and Pl_{16}, are positioned to the lower end and linked to a common marker, HT636, and Pl_{14} is associated with a tandemly duplicated cluster of genes encoding the non-Toll/interleukin receptor (TIR)-like NBS-LRR (Mulpuri et al. 2009; Bachlava et al. 2011; Liu et al. 2012). It is unknown whether these genes are either tightly linked or constitute different alleles of the same locus. However, Pl_{ARG} in LG1 was mapped to a recombinationally suppressed region located far from other *Pl* genes in LG1 (Dußle et al. 2004; Wieckhorst et al. 2010). Different from LG1, five *Pl* genes (Pl_1–Pl_2–Pl_6–Pl_7–Pl_{15}) and one rust resistance gene (R_1) are clustered on LG8 (Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Brahm et al. 2000; Gedil et al. 2001; Bouzidi et al. 2002; Slabaugh et al. 2003; Radwan et al. 2008; de Romano et al. 2010; Franchel et al. 2013). ORS166, an SSR marker that has been reported to be linked to Pl_{15} and the Pl_1–Pl_2–Pl_6–Pl_7 cluster, was mapped to 9.7 cM in the sunflower C-Map with 36 co-segregating RGC (resistance gene candidate) markers (http://www.sunflower.uga.edu/cmap/). The TIR NBS-LRR sub-cluster was found in the LG8 cluster of resistance genes (Bouzidi et al. 2002; Franchel et al. 2013).

In the lower end of LG13, there are seven resistance genes consisting of three *Pl* genes (Pl_5/Pl_8 and Pl_{21}) and four rust resistance genes (R_{Adv}, R_4, R_{11}, and R_{13}), plus two male fertility restoration genes (*Rf1* and *Rf5*) (Gentzbittel et al. 1995; Lawson et al. 1998; Bert et al. 2001; Horn et al. 2003; Radwan et al. 2003, 2004; Yu et al. 2003; Yue et al. 2010; Bachlava et al. 2011; Qi et al. 2011, 2012; Vincourt et al. 2012; Gong et al. 2013). This gene cluster in LG13 is divided into two sub-clusters. Sub-cluster I includes two rust resistance genes (R_{Adv} and R_{11}) and two male fertility restoration genes (*Rf1* and *Rf5*), and sub-cluster II has three *Pl* genes (Pl_5/Pl_8 and Pl_{21}) and two rust resistance genes (R_4 and R_{13}). Pl_5/Pl_8 and R_4/R_{13} were placed between two RGC markers, RGC15/16 and RGC251, whereas Pl_{21} was 8 cM away from Pl_5 (Vincourt et al. 2012; Gong et al. 2013). Bachlava et al. (2011) identified the novel non-TIR-like NBS-LRR RGCs associated with Pl_8, which clustered with previously identified RGCs on LG13 but were phylogenetically distant from the TIR- and non-TIR NBS-LRR classes.

Although Pl_{17} is the only DM resistance gene in LG4, it also resides near an RGC cluster (Fig. 2, Radwan et al. 2008). It would be interesting to know the differences between the gene structures of Pl_{17} and the other clustered *Pl* genes, and positional cloning of the functional Pl_{17} gene would elucidate a genetic mechanism by which Pl_{17} was involved in the evolution of sunflower *Pl* genes. Identification of the molecular markers flanking Pl_{17} in the present study is a starting point for map-based cloning of this gene. The question of whether a gene is located in a region with a high or low recombination rate is essential information for the map-based cloning of sunflower genes. The consensus map of the sunflower genome is 1,443.8 cM and was constructed from three F_2 populations of HA 89/RHA 464, B-line/RHA 464, and CR 29/RHA 468 (Talukder et al. 2014), while the sunflower haploid genome consists of ~3.5 Gb (Baack et al. 2005). Thus, in sunflowers, 1 cM is approximately equivalent to 2.4 Mb at the whole genome level. The genetic distance on a linkage map is the product of genetic recombination. The ratio of Mb/cM may vary across the genome and within a chromosome, as the recombination rate is not constant along the length of the chromosome and most recombination events occur at highly localized hot spots. In wheat, it was reported that the kilobase pairs per centimorgan ratio was approximately 110-fold greater in a region of low recombination (22 Mb/cM) compared to a recombination hot spot (118 kb/cM) (Gill et al. 1996; Faris et al. 2000). The non-random physical distribution of recombination events has also been reported in other plants (Tanksley et al. 1992; Schmidt et al. 1995; Künzel et al. 2000). In the present study, the Pl_{17} gene was located in a region with the recombination frequency of 0.59 Mb/cM, a fourfold increase in recombination compared to the genomic average. Two flanking SNP markers delineate a region of 2,251,471 bp that contains Pl_{17}. The closest marker, ORS963, is 0.9 cM (approximately 0.53 Mb) away from Pl_{17}. This region can be considered to be amenable to molecular manipulation, and a search for new molecular markers to use in the further refinement of the interval containing Pl_{17} is underway using the genomic sequence information available at http://sunflowergenome.org/early_access/repository/main/genome_browser/blast.html.

Sunflower downy mildew is a highly virulent and aggressive disease with a great potential for developing new races. In the 1970s, only two races, 100 and 300 (then referred to as races 1 and 2), were reported in Europe and North America (Zimmer 1974). The continuous use of sunflower hybrids with different resistance genes has led to a highly diverse population of *P. halstedii* in the

sunflower production regions around the world, and 36 races of *P. halstedii* have been identified as of 2006 (Tourvieille de Labrouhe et al. 2000; Gulya 2007; Gulya et al. 2011; Viranyi 2008). The increasing number of new *P. halstedii* races overcomes the resistance mediated by *Pl* genes. In 1988, *P. halstedii* races 710 and 703 were found to be virulent against the Pl_1 and Pl_2 genes, which have been deployed in sunflower production since 1978 (Tourvieille de Labrouhe et al. 1991). After another decade, Pl_6 and Pl_7 were overcome by several new races of *P. halstedii* in North America and France (Tourvieille de Labrouhe et al. 2000; Gulya 2007; Gulya et al. 2011). Although there has been no record of races overcoming Pl_{17} in HA 458 (Gulya et al. 2011), pyramiding strategies must be considered for the deployment of a durable resistance and Pl_{17} should be used in combination with other *Pl* genes that are either race specific or non-race specific. Robust DNA markers are required to facilitate this breeding approach. Such markers are now available for a number of *Pl* genes, including Pl_8 and Pl_{ARG}, which are still effective against all races of *P. halstedii* (Dußle et al. 2004; Wieckhorst et al. 2010; Radwan et al. 2003, 2004; Bachlava et al. 2011; Gulya et al. 2011). The markers identified here will be particularly useful in pyramiding Pl_{17} with different *Pl* genes that cannot be distinguished in downy mildew bioassays, thereby helping to achieve durable control of downy mildew in sunflowers.

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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.

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