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ORIGINAL PAPER

Inheritance and molecular mapping of a downy mildew resistance gene, Pl_{13} in cultivated sunflower (*Helianthus annuus* L.)

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Abstract The inheritance of resistance to sunflower downy mildew (SDM) derived from HA-R5 conferring resistance to nine races of the pathogen has been determined and the new source has been designated as Pl_{13}. The F_2 individuals and F_3 families of the cross HA-R5 (resistant) × HA 821 (susceptible) were screened against the four predominant SDM races 300, 700, 730, and 770 in separate tests which indicated dominant control by a single locus or a cluster of tightly linked genes. Bulked segregant analysis (BSA) was carried out on 116 F_2 individuals with 500 SSR primer pairs that resulted in the identification of 10 SSR markers of linkage groups 1 (9 markers) and 10 (1 marker) of the genetic map (Tang et al. in Theor Appl Genet 105:1124–1136, 2002) that distinguished the bulks. Of these, the SSR marker ORS 1008 of linkage group 10 was tightly linked (0.9 cM) to the Pl_{13} gene. Genotyping the F_2 population and linkage analysis with 20 polymorphic primer pairs located on linkage group 10 failed to show linkage of the markers with downy mildew resistance and the ORS 1008 marker. Nevertheless, validation of polymorphic SSR markers of linkage group 1 along with six RFLP-based STS markers of linkage group 12 of the RFLP map of Jan et al. (Theor Appl Genet 96:15–22, 1998) corresponding to linkage group 1 of the SSR map, mapped seven SSR markers (ORS 965-1, ORS 965-2, ORS 959, ORS 371, ORS 716, and ORS 605) including ORS 1008 and one STS marker (STS10D6) to linkage group 1 covering a genetic distance of 65.0 cM. The Pl_{13} gene, as a different source with its location on linkage group 1, was flanked by ORS 1008 on one side at a distance of 0.9 cM and ORS 965-1 on another side at a distance of 5.8 cM. These closely linked markers to the Pl_{13} gene provide a valuable basis for marker-assisted selection in sunflower breeding programs.

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Introduction

Downy mildew incited by *Plasmopara halstedii* (Farl.) Berl. et de Toni, is a major disease in all sunflower growing regions (Sackston 1981). It is a seed-, air- and soil-borne disease that often damages more than 50% of the plants in an affected field and accounts for more than 80% loss of potential production (Molinero-Ruiz et al. 2003). Among the various methods of control, host-plant resistance using race-specific resistance genes, designated as *Pl* genes, is the most effective. Sources of resistance to downy mildew have been obtained from wild *H. annuus* L., *H. praecox* Englm. and A Gray, *H. tuberosus* L. and *H. argophyllus* T. and G. (Jan et al. 1991; Miller and Gulya 1991; Pustovoit and Krokhin 1977; Rahim et al. 2002; Vear 1974; Vranceanu and Stoenescu 1970; Zimmer and Kinman 1972). The predominant races of downy mildew in the major sunflower producing countries are 700, 703, 710, 730, and 770. Commercial hybrids using USDA-ARS lines HA 335 and

HA 336 (Pl_6), HA 337, HA 338, and HA 339 (Pl_7) and RHA 340 (Pl_8) are resistant to all *P. halstedii* races known to exist in North America (Gulya 2007). Commercial hybrids based on HA 335, HA 337 and HA 338 give resistance to races 703 "(*was race B*)" and 710 "(*was race A*)" in France. The effectiveness of the major resistance genes (Pl_1, Pl_2 and Pl_6) has been overcome by new races in France (Delmotte et al. 2008); however, there are no records of races overcoming other broad spectrum (Pl_8, and Pl_{Arg}) genes (Gulya 2007). Pl_7 has exactly the same race reaction as Pl_6 and both are susceptible to races 304, 314, 307, 704, and 714 (Vear 2004). There is a constant evolution of new physiological races due to pathogenic variability and selection pressure resulting from the use of resistant hybrids and seed treatment fungicides that continuously challenges the breeders to identify new resistance sources. To date, 36 physiological races and 18 *Pl* genes have been described (Gulya 2007; Molinero-Ruiz et al. 2003; Rahim et al. 2002). Sources of the *Pl* genes and their resistance to different pathotypes have been reviewed by Rahim et al. (2002). While some of the sources have been genetically characterized, only a few loci (Pl_1, Pl_2, Pl_5, Pl_6, Pl_7, Pl_8, Pl_{Arg}) have been associated with molecular markers.

Several hypotheses have been proposed regarding the mode of action of genes conferring resistance to downy mildew in sunflower, with most studies indicating the control of resistance by major dominant genes (Miller and Gulya 1987, 1991; Molinero-Ruiz et al. 2003; Tan et al. 1992; Vear 1974; Zimmer and Kinman 1972). Inheritance studies indicated that some downy mildew resistance genes could be linked, i.e., Pl_2 and Pl_4 (Sackston 1981), Pl_1 and Pl_2 (Mouzeyar et al. 1995), Pl_1 and Pl_6 (Roeckel-Drevet et al. 1996), Pl_1, Pl_2 and Pl_6 (Vear et al. 1997). Studies by Vear et al. (1997) indicated that the Pl_6 "locus" (a cluster of Pl_1, Pl_2, and Pl_6) is split into at least two genetically distinct regions: one region with resistance to races 100 and 300 and another region with resistance to races 700, 703 and 710. Rahim et al. (2002) characterized the Pl_{12} resistance gene and determined the inheritance and allelic relationships of genes conferring resistance to races 100, 300, and 700. It was postulated that the resistant loci contain several copies of conserved genes of the TIR-NBS-LRR class of plant resistance genes (Bouzidi et al. 2002).

Molecular markers linked to genes conferring resistance to different races of downy mildew in sunflower have been identified and mapped using mostly the bulked segregant analysis method (Michelmore et al. 1991), with the exception of the study of Pankovic et al. (2007) where NILs were utilized. The Pl_1 locus was mapped using two RFLP markers and a RAPD marker (Mouzeyar et al. 1995). Bert et al. (2001) mapped the Pl_5 locus from the Russian cultivar 'Progress' to linkage group 6 of the consensus RFLP map of Gentzbittel et al. (1995) which corresponds to linkage group 13 of the genetic map of Yu et al. (2003). The Pl_5/Pl_8 locus conferring resistance to a wide range of *P. halstedii* races was mapped using STS markers designed from R-gene analogs to the same linkage group (Radwan et al. 2004). The Pl_6 locus in the inbred line HA 335 was mapped on to the linkage group 1 of the consensus RFLP map (Roeckel-Drevet et al. 1996; Vear et al. 1997). Pankovic et al. (2007) mapped Pl_6 onto linkage group 8 of the SSR map (Yu et al. 2003). Bert et al. (2001) reported two downy mildew resistance genes on linkage groups 8 and 13. Genes for resistance to downy mildew races 1 (100, Pl_1) and D (300, Pl_2) were linked to Pl_6 (Vear et al. 1997) and reside on linkage group 8 (Gedil et al. 2001). Mapping of the Pl_{Arg} locus with SSR markers indicated its location on linkage group 1 (Duble et al. 2004). These results suggest that inheritance of resistance to downy mildew in sunflower varies with the genetic background and could be either allelic or independent.

HA-R5 is a germplasm with resistance to four races of rust (*Puccinia helianthi* Schwein), nine races of downy mildew, and verticillium wilt (*Verticilium dahliae* Klebahnis) and can facilitate the incorporation of resistance to three major diseases from a single source (Gulya 1985). The present study investigates the inheritance of resistance of HA-R5 to four predominant races of downy mildew (300, 700, 730, and 770) and maps the resistance gene onto the sunflower linkage map using an F_2 population derived from a cross between HA-R5 (resistant) and HA 821 (susceptible).

Materials and methods

Plant materials and mapping populations

HA-R5 is a composite germplasm derived from an Argentine open-pollinated variety, Guayacan, from INTA. This germplasm was originally released for its resistance to the four races of rust (1, 2, 3, and 4) indigenous to North America, but it also is resistant to downy mildew and verticillium wilt (Gulya 1985). Subsequently, it was found to confer resistance to races 100, 300, 310, 330, 700, 710, 730, 731, and 770 but susceptible to races 307 and 703 and is being used as a differential line for downy mildew race determination in the USA (Gulya et al. 1998) and was the source of the differential line D8 (QHP1) in France (Vear et al. 2008a). Here, for convenience, we tentatively designated the resistance gene from HA-R5 as Pl_{13}. HA 821 is an oilseed germplasm with mid-season maturity. It is moderately susceptible to rust and verticillium wilt and susceptible to all known races of downy mildew (Roath et al. 1986). Crosses were made between HA-R5 and HA 821 and DNA samples were obtained from 116 F_2 plants. The F_2 individuals

were self-pollinated to obtain F_3 seeds, which were used for the resistance test to confirm the F_2 genotype.

Screening for resistance to downy mildew

Spores of *P. halstedii* races 300, 700, 730, and 770 were stored in liquid nitrogen. Each vial of frozen spores was heat-shocked in 60°C water for 30 s, then suspended in non-chlorinated spring water (amended with 13 ml of 2 M $CaCl_2$/3.78 L spring water) and used to inoculate a universal susceptible line, HA 89, for spore increase. Following appearance of disease symptoms on these seedlings, the freshly produced zoosporangia were used to inoculate seedlings of the nine differential lines for confirmation of the races (Gulya et al. 1998).

Disease reaction of the F_2 individuals and F_3 families was determined separately for each of the four races. Using the whole seedling immersion technique (Rahim et al. 2002), the 116 F_3 families were screened separately for reaction to downy mildew races 300, 700, 730, and 770 to confirm their corresponding F_2 genotypes. In addition, four F_2 populations with 116, 145, 147, and 177 plants were also screened separately for the above four races.

The seeds were sterilized with 2% sodium hypochlorite for 10 min, thoroughly rinsed with tap water, placed between moist germination blotters and incubated in a germinator at 26 ± 2°C for 2–3 days until the radicles were 1–2 cm long with visible root hairs. About 100–180 F_2 seedlings and 25–35 seedlings of each F_3 family were inoculated with each of the four races using an inoculum load of 2×10^4 freshly harvested zoosporangia/ml for 3 h at 18°C. The inoculated seedlings were planted in flats with a 3:2 coarse sand:perlite mixture and grown for 11 days in the greenhouse (25°C, 16-h photoperiod) and then transferred to a humidity chamber maintained at 18°C and 100% relative humidity for 16 h for sporulation. Each inoculation treatment included the parents and three differential lines, RHA 274, DM-2 and 803-1. RHA 274 is resistant to race 300 and susceptible to races 700, 730 and 770. DM-2 is resistant to races 300 and 700 and susceptible to 730 and 770, while 803-1 is resistant to races 300, 700, 730 and susceptible to race 770. All the three differential lines are susceptible to race 770. For races 730 and 770, seedlings were scored as susceptible if fungal sporulation was observed on all the leaves. However, in case of races 300 and 700 which are less aggressive than the other two races, seedlings with fungal sporulation only on the cotyledons or just a miniscule tuft of zoosporangia on the cotyledons were also scored as susceptible. Among the tested races, race 300 was the least aggressive and hence, the inoculated seedlings were resporulated for another week. Based on the reaction of the F_3 families, the F_2 individuals were categorized as homozygous and heterozygous resistant and homozygous susceptible plants.

DNA extraction

Genomic DNA was extracted from the parents (HA-R5 and HA 821), F_1 and the F_2 plants according to the protocol of Rogers and Bendich (1985). Freeze dried (−80°C) and lyophilized leaves were ground to a fine powder and used for DNA extraction. The concentration of DNA was quantified using an Eppendorf biophotometer (Hamburg, Germany) and adjusted to 30 ng/µl for PCR amplification. Bulks of resistant and susceptible individuals were constituted by pooling equal quantities of DNA from 12 homozygous resistant and susceptible F_2 plants for screening polymorphism between bulks using the bulked segregant analysis method (Michelmore et al. 1991).

PCR analysis

Five hundred mapped sunflower specific SSRs from the Compositae database (http://compositdb.ucdavis.edu) spanning the 17 linkage groups were screened for polymorphism between the parental lines and the resistant and susceptible bulks. The primers that showed polymorphism between the bulks were tested on 12 plants that constituted the resistant and susceptible bulks. The primers that generated reproducible polymorphism and with less than 50% recombinants in individual plant assays were selected for genotyping the 116 F_2 individuals.

PCR amplification was conducted according to Tang et al. (2002) with minor modifications. The 15-µl PCR reaction mixture contained 1.5 µl of 10× PCR buffer, 0.3 µl of 25 mM $MgCl_2$, 1.2 µl of 2.5 µM dNTPs, 0.4 µl containing 10 pmol of the forward and reverse primers, 2 µl of 30 ng/µl DNA and 0.2 µl (5 units/µl) *Taq* DNA polymerase (Qiagen, Valencia, CA, USA). PCR amplifications were performed using the "touchdown" profile (Feng and Jan 2008) to minimize nonspecific amplification in an MJ Research single (Watertown, MA, USA) or a Bio-Rad dual 96-well thermal cycler (Hercules, CA, USA). The amplified products were denatured at 95°C for 5 min and electrophoresed on a 6.5% denaturing polyacrylamide gel at 60 W for 2.0 h (1× TBE) on a CBP Scientific gel electrophoresis system. The gels were stained with GelRed nucleic acid gel stain (Biotium Inc, CA, USA) and scanned with a Typhoon 9410 variable mode imager (Molecular Dynamics Inc., CA, USA).

Six single- or low-copy RFLP markers from linkage groups 12 and 16 (Jan et al. 1998) which cross-referenced to linkage groups 1 and 10, respectively, of the SSR genetic map (Yu et al. 2003) were also selected to design sequence tagged sites (STS) primers to screen for additional polymorphic primers. The primers were designed using Primer 3 software (http://biotools.unassmed.edu/bioapps/primer3_www.cgi). Of these six STS primer pairs, one primer pair

generated linked polymorphic marker, and the sequences of this STS primer pair is as follows: STS10D6, 5′-AACTAC GACCCACAAAAGGACAAG-3′, and 5′-TTAGACCAG GGCCCAACAAAC-3′. The PCR amplification profile included a hot start at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s and extension at 72°C for 90 s with a final extension at 72°C for 10 min. Amplified products were run on 6.5% denatured polyacrylamide gels.

Statistical analysis

Chi-square test was performed for reaction to downy mildew of the F_2 individuals and F_3 families and also on each marker locus for detection of deviations from the expected Mendelian ratios for dominant (3:1) and codominant (1:2:1) markers in the F_2 generation. Data from downy mildew phenotyping and molecular marker analysis with SSR and RFLP-STS were subjected to linkage analysis using the MAPMAKER/Exp version 3.0b program (Whitehead Institute, Cambridge, MA, USA) (Lander et al. 1987) and a partial linkage map of the region on the chromosome surrounding the Pl_{13} gene was constructed. Markers were sorted in linkage groups using the two-point analysis "group" command and the most likely order was determined by using the "compare" command. Marker grouping and map order was based on maximum-likelihood estimates with a constant LOD score of 3.0 and a maximum recombination frequency of 0.50. The "error detection on" command was used to check for errors in scoring and data entry, and for double crossover events. The recombination fractions were converted to genetic distances in centi-Morgan (cM) using the Kosambi mapping function (Kosambi 1944). The linkage maps were drawn using the MapChart software (Voorrips 2002).

Results

All plants of the resistant parent (HA-R5) were resistant to the four races (300, 700, 730, and 770) of downy mildew, while the susceptible parent (HA 821) had 12, 8, 3 and 0% non-infected plants to races 300, 700, 730, and 770, respectively. The segregation ratio of the F_2 generation for reaction to the four races of downy mildew fits a 3 resistant:1 susceptible Mendelian ratio (Table 1). The F_2 population tested for 300 and 700 tend to have a slightly higher frequency of resistant plants due to the weak aggressiveness of these two races. However, their segregation remained within the expected 3 resistant:1 susceptible ratio. The F_3 families of the cross HA-R5 × HA 821 reacted uniformly to the 4 races with no recombinants observed. Of the 116 F_3 families, 29 were homozygous resistant, 62 were heterozygous resistant and 25 were homozygous susceptible. This segregation ratio of resistance to susceptible fit the Mendelian segregation ratio of 1:2:1 ($\chi^2 = 0.828$, $P = 0.66$) and suggested that downy mildew resistance in HA-R5 is controlled by a single dominant gene or a cluster of several tightly linked genes.

Of the 500 SSR primer pairs tested, 213 generated polymorphic bands resulting in a 42.6% polymorphism between HA-R5 and HA 821. Assessment of the 213 polymorphic primers on resistant and susceptible bulks and F_2 population resulted in identification of seven SSR markers, including one marker from linkage group 10 (ORS 1008) and six markers from linkage group 1 (ORS 965-1, ORS 965-2, ORS 959, ORS 371, ORS 605, ORS 716) associated with the downy mildew resistance phenotype. Of these, ORS 1008 and ORS 965 were the markers closest to the Pl_{13} locus. ORS 1008 (Fig. 1) was multilocus and produced a polymorphic dominant band; and ORS 965 produced two polymorphic markers of which one is dominant and the other a codominant marker.

Because ORS 1008 is located on linkage group 10 of the SSR maps (Tang et al. 2002; 2003), 20 polymorphic SSR markers (ORS 3, 78, 363, 380, 433, 437, 537, 541, 591, 682, 705, 708, 779, 815, 849, 853, 878, 1048, 1112, 1130) on linkage group 10, including several markers that co-segregate with ORS 1008, and six RFLP-based STS markers from linkage group 10 (STS7A2, 8A1, 8B4, 10B3, 11E2 and 15B3), were screened on the 116 F_2 individuals. Linkage analysis failed to link any of the above markers to the

Table 1 Segregation of the Pl_{13} locus for resistance to the four races of downy mildew in the F_2 and F_3 generations of the cross HA-R5 × HA 821

Generation	Race(s) screened	Observed frequency			Ratio tested	χ^2	P
		Resistant[a]		Susceptible			
		RR	Rr	rr			
F_2	300	112		33	3:1	0.581	0.45
	700	96		20	3:1	3.724	0.05
	730	138		39	3:1	0.756	0.38
	770	108		39	3:1	0.144	0.70
F_3 family[a]	300, 700, 730, 770	29	62	25	1:2:1	0.828	0.66

[a] The reaction to all the four races was similar and the number of F_3 families phenotyped was the same (116); hence, the phenotypes were grouped

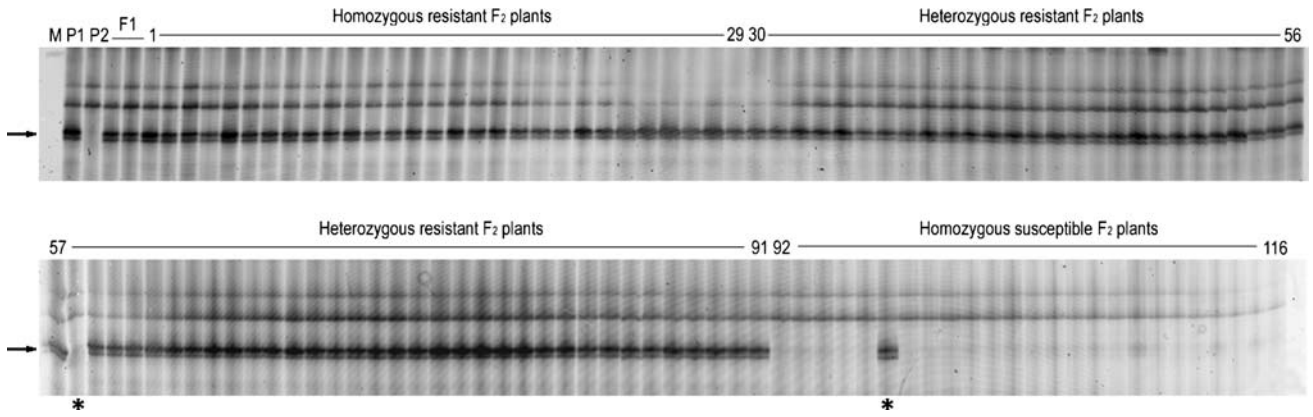

Fig. 1 Genotyping of the 116 F_2 individuals derived from the cross between HA-R5 × HA 821 along with parents (*P1* resistant parent HA-R5, *P2* susceptible parent HA 821) and F_1s with ORS 1008. *Arrow* indicates the marker scored. *M* indicates 100-bp DNA ladder G210A (Promega, Madison, WL, USA); *asterisk* represents recombinants

Table 2 Segregation of the seven SSR markers and one STS marker linked to Pl_{13} locus in an F_2 population from HA-R5 × HA 821

Marker	Observed frequency[a]				Ratio tested	χ^2	*P*
	A	H	B	D			
ORS 1008			25	91	1:3	0.736	0.39
ORS 965-1			24	92	1:3	1.149	0.28
ORS 371	37	57	22		1:2:1	3.914	0.14
STS10D6	37	55	24		1:2:1	3.224	0.20
ORS 959	36	57	23		1:2:1	2.948	0.23
ORS 716	35	55	26		1:2:1	1.707	0.43
ORS 965-2	37	58	21		1:2:1	4.414	0.11
ORS 605	37	49	30		1:2:1	3.638	0.16

[a] Genotypes: *A,* HA-R5 (*RR*); *H,* heterozygous (*Rr*); *B,* HA 821 (*rr*); *D,* not B (*RR* or *Rr*)

ORS 1008 or the Pl_{13} locus. These results indicated that the gene for resistance to downy mildew in HA-R5 is not located on linkage group 10.

Subsequently, RFLP-based STS markers (STS1A6, 2A1, 7A4, 9E2, 10D6, and 21D2) on linkage group 12 of the RFLP map (Jan et al. 1998) corresponding to linkage group 1 of the SSR map were tested. Of these, STS21D2 (dominant) and STS10D6 (codominant) detected polymorphism between parents. Validation of these two markers on the 116 F_2 individuals linked STS10D6 to the Pl_{13} gene.

The segregation ratio of the six linked SSR markers and one RFLP-based STS marker was in agreement with Mendelian segregation ratios of 1:2:1 and 3:1 for codominant and dominant markers, respectively (Table 2). The Pl_{13} gene was flanked by ORS 1008 on one side at a distance of 0.9 cM and ORS 965-1 on another side at a distance of 5.8 cM. The other six markers were loosely linked with the Pl_{13} gene. The final map was constructed using the SSR and STS markers covering a distance of 65.0 cM (Fig. 2a). Since the order of all the SSR markers (except ORS 1008) linked to Pl_{13} is similar to linkage group 1 of the SSR map (Tang et al. 2003), it can be concluded that the Pl_{13} gene is located on linkage group 1 (Fig. 2b).

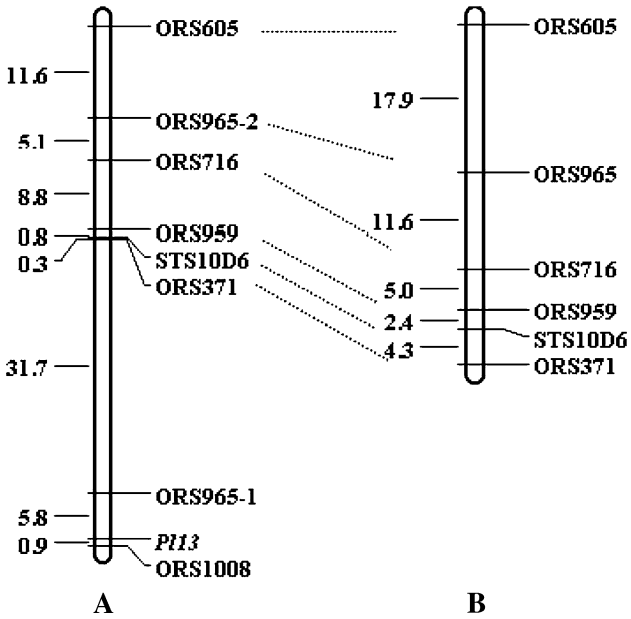

Fig. 2 Pl_{13} genetic map showing the location of the Pl_{13} locus on linkage group 1 of the SSR map and linked to SSR and STS markers. **a** Linkage map for the Pl_{13} region based on the analysis of 116 F_2 plants derived from the cross between the resistant line HA-R5 and the susceptible line HA 821. **b** Corresponding region of linkage group 1 of the SSR map (Tang et al. 2003). Genetic distance of STS10D6 is determined from the saturated map of Yu et al. (2003). The distances are given in centiMorgan (cM)

Discussion

Resistance to downy mildew controlled by dominant *Pl* genes has been successfully transferred to suitable agronomic backgrounds. However, evolution of physiological

races of the pathogen that overcome widely used resistant genes is a continuous process necessitating the identification of new broad spectrum sources of resistance. Resistance genes identified could confer resistance to a single race, two races or multiple races and is controlled by single dominant gene (Miller and Gulya 1987; 1991; Molinero-Ruiz et al. 2003; Zimmer and Kinman 1972; Vranceanu et al. 1981; Pustovoit and Krokhin 1977; Vranceanu and Stoenescu 1970; Tan et al. 1992), digenic (Molinero-Ruiz et al. 2002; Rahim et al. 2002), digenic regulated by complementary and epistatic relationships (Molinero-Ruiz et al. 2002), a cluster of genes (Bouzidi et al. 2002; Radwan et al. 2003; Slabaugh et al. 2003; Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997), or quantitatively inherited (Vear et al. 2008b). Eighteen genes (*Pl*$_1$–*Pl*$_{12}$, *Pl*$_{Arg}$ and *Plv* to *Plz*) have been proposed to confer resistance to different races of downy mildew in sunflower (Vranceanu and Stoenescu 1970; Vear and Leclercq 1971; Zimmer and Kinman 1972; Leclercq et al. 1970; Jan et al. 1991; Pustovoit et al. 1976; Vranceanu et al. 1981; Miller and Gulya 1987; 1991; Tan et al. 1992; Fick et al. 1975; Gulya et al. 1991; Molinero-Ruiz et al. 2002; 2003; Rahim et al. 2002; Duble et al. 2004). The resistance gene identified in HA-R5 conferring resistance to a broad spectrum of races (100, 300, 310, 330, 700, 710, 730, 731, and 770) is monogenic and dominant.

In this study, the F_3 population derived from the cross HA-R5 × HA 821 was screened separately against four predominant races (300, 700, 730, and 770) of downy mildew, and genetic control by a single, dominant locus was indicated. However, due to the limited population size of this study, we cannot rule out the possibility of the control of resistance by a cluster of several tightly linked genes. Roeckel-Drevet et al. (1996) also carried out separate tests with French races 703 and 710. They reported that the *Pl*$_{Arg}$ was reported to confer resistance to all known races of downy mildew, but screening of the F_2 progeny for mapping was done with only one race (730) (Duble et al. 2004). Likewise, the population for mapping the *Pl*$_6$ gene was subjected to screening for downy mildew resistance against race 730 (Pankovic et al. 2007). Vear et al. (1997) emphasized the need to characterize different sources of resistance with all races to determine if they have different resistance genes. They clearly demonstrated the recombination between loci and the existence of at least two distinct loci with one governing resistance to races 100 and 300 and the second conferring resistance to races 700, 703, and 710.

Of the markers linked to *Pl*$_{13}$, ORS 1008 was the closest marker at a genetic distance of 0.9 cM. Linkage group 10 is a dense linkage group and has the most number of SSR and EST markers (Tang et al. 2003; Lai et al. 2005). Despite the availability of the maximum number (28) of single-locus SSR markers on linkage group 10 (Tang et al. 2003), it could not be mapped to the respective linkage group when genotyped with 20 SSR markers from this linkage group. The primer ORS 1008 produced multiple products and distinguished the resistant from susceptible genotypes by a dominant allele (Fig. 1). It is rare to have dominant RFLP and SSR markers, but in sunflower 11–35% of the RFLP probes showed dominant segregation patterns and 30% of the probes detected duplicated loci (Berry et al. 1995; Gentzbittel et al. 1995; Jan et al. 1998). Some of the public SSR markers developed for sunflower produce null alleles and amplify multiple loci, while others amplify a single locus and are codominant (Tang et al. 2002, 2003; Yu et al. 2003). ORS 1008 represents a multilocus, dominant SSR marker (Tang et al. 2003). Interestingly, the bacterial artificial chromosome (BAC) clones sharing the ORS 1008 marker were placed in four different contigs confirming the multilocus nature of the marker (Bouzidi et al. 2006). Likewise, ORS 965 is also a multilocus marker (Tang et al. 2003). It is possible that the polymorphic markers scored in RHA 280 × RHA 801 (Tang et al. 2002, 2003) could be different from the two new markers (ORS 1008 and ORS 965-1) scored in HA-R5 × HA 821 population used in the present study.

The *Pl*$_{13}$ locus is mapped at the proximal end of linkage group 1 (Fig. 2). SSR markers are lacking in the region after ORS 552 in linkage group 1. The markers available to cover the gap of 37.5 cM between ORS 371 and *Pl*$_{13}$ are the RFLP marker, ZVG4-1 located at a distance 35.3 cM (Berry et al. 1995) and the TRAP markers (Yue et al. 2008), which could be used to confirm the 30-cM linkage with other markers on linkage group 1 to confirm the position of *Pl*$_{13}$. The map order obtained in relation to *Pl*$_{13}$ locus was conserved and similar to that of the genetic map of Tang et al. (2003) and Yu et al. (2003). Minor variations in genetic distances among the markers were observed. However, the deviations are not significant and could possibly depend on the population and the individuals genotyped.

A major cluster harboring the *Pl*$_1$, *Pl*$_2$, *Pl*$_5$ and *Pl*$_6$ genes has been located on linkage group 1 of the consensus RFLP linkage map of Gentzbittel et al. (1995) which corresponds to linkage group 8 of the SSR linkage map of Yu et al. (2003) (Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997). A second major locus (linkage group 13) was identified that contained two clustered resistance genes *Pl*$_5$ and *Pl*$_8$ (Bert et al. 2001; Radwan et al. 2004). The *Pl*$_{Arg}$ and *Pl*$_8$ derived from different *H. argophyllus* sources, ARG 1575-1 and ARG 415, respectively, were mapped on different linkage groups (linkage group 1 and linkage group 13) (Duble et al. 2004; Radwan et al. 2004). The *Pl*$_{13}$ gene in the present investigation was mapped onto linkage group 1 of Tang et al. (2003). Mapping of STS10D6 along with the SSR markers on linkage group 1 is in agreement with

the previous maps (Jan et al. 1998; Yu et al. 2003) and confirms the location of the Pl_{13} gene on linkage group 1.

The only other *Pl* gene that was mapped to linkage group 1 on the SSR map, Pl_{Arg} is 1.9 cM from the marker ORS 662 (Duble et al. 2004). However, Pl_{13} and Pl_{Arg} were located at different loci. While Pl_{13} was mapped proximal to ORS 371, Pl_{Arg} was mapped distal to the same marker. Therefore, because the Pl_{13} in HA-R5 is different from Pl_1 to Pl_{12}, and Pl_{Arg}, it is reasonable to designate this gene as Pl_{13}. Pl_{13} has flanking markers on both sides of the gene, while the Pl_{Arg} locus has no markers distal to the resistance gene. The efficiency of MAS is increased by employing markers flanking the target locus rather than with the use of a single marker (Chahal and Gosal 2002). With regard to Pl_{Arg}, there was significant segregation distortion for phenotypic resistance evaluation and molecular markers besides significant reduction (15%) in map length (Duble et al. 2004). However, such distortions in marker segregation patterns and deviations in map length were not evident with the mapping of the Pl_{13} locus. Identification of closely linked markers on both sides of the Pl_{13} gene will help in selection of recombinants for the Pl_{13} locus. Nevertheless, allelism tests need to be performed to determine relationships between Pl_{13} and other *Pl* genes located on the same linkage group.

The molecular markers identified in the present study will facilitate the marker-assisted selection program aimed at incorporation of downy mildew resistance from HA-R5. Markers closely linked to *Pl* genes provide a reliable approach for identification of lines carrying the resistance gene, precluding the need of progeny testing. While the value of closely linked dominant markers cannot be underestimated, codominant, single-locus markers will be helpful in distinguishing homozygous and heterozygous individuals and mapping phenotypic traits. The SSR linkage map proximal to ORS 371 is sparse; however, publicly accessible SNP, EST-SSR and INDEL databases are available (Pashley et al. 2006; Heesacker et al. 2008). Tang et al. (2003) emphasized the importance of multilocus and dominant SSR markers for phenotypic and quantitative trait loci residing in regions lacking single-locus SSR marker loci which could supply a significant number of genomic landmarks for isolating BAC clones. Three BAC libraries are available (Gentzbittel et al. 2002; Bouzidi et al. 2006; Feng et al. 2006) which could be screened to generate additional markers linked to downy mildew resistance in HA-R5.

The HA-R5 germplasm is a proven source of resistance to both downy mildew and rust. Many inbred lines possessing resistance to downy mildew possess resistance to rust as well (Cheres and Knapp 1998; Vranceanu and Stoenescu 1970; Fick and Zimmer 1975; Gedil et al. 2001). Studies of Slabaugh et al. (2003) using RGCs substantiated the linkage of rust resistance and downy mildew resistance on linkage group 8 of the SSR map of Yu et al. (2002). Subsequently, Yu et al. (2003) found duplicated genes for resistance to rust in close proximity to duplicated genes for resistance to downy mildew on linkage groups 8 and 13. Sunflower is reported to have genomic organization of a region on linkage group 8 where genes active against downy mildew and rust pathogens reside (Bouzidi et al. 2002; Gentzbittel et al. 1998; Mouzeyar et al. 1995; Gedil et al. 2001; Vear et al. 1997). However, comparison of phenotypic data available for the F_2 and F_3 population of the HA-R5 × HA 821 cross for rust and downy mildew, respectively, indicate that the genes conferring resistance to these two major diseases are not allelic (Jan, unpublished). Linkage of Pl_{13} gene to the genes conferring resistance to the four races of rust has not been determined.

The Pl_{13} could be a useful source of resistance to the four major races of downy mildew (races 300, 700, 730, and 770) and can be successfully transferred to different genetic backgrounds. The identified markers closely linked to downy mildew resistance are expected to greatly enhance the efficiency of breeding using marker-assisted selection. Ongoing research towards the development of RILs from the cross HA-R5 × HA 821 will attempt to map genes conditioning rust and verticillium wilt resistance.

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