

Molecular Mapping of Downy Mildew and Rust Resistance Genes in TX16R

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Abstract

Inbred line TX16R is homozygously resistant to at least five sunflower downy mildew races and 11 rust races. This study reports the mapping of the downy mildew resistance gene PI_{TX16R} and the rust resistance gene R_{TX16R} using an F_2 population derived from HA 434 x TX16R. Progeny test of half-diallel test crosses to downy mildew suggested that PI_{TX16R} localizes on the same linkage group (LG) 4 with PI_{17} from HA 458. Molecular mapping of PI_{TX16R} identified a co-segregating marker, ORS963, and a tightly linked marker ORS644 to PI_{TX16R} at a genetic distance of 0.3 cM on the distal side, with seven markers covering a genetic distance of 22.9 cM on LG 4. This is the second PI gene reported on LG 4. Bulk segregant analysis with SSR primers and STS primers from the LGs with previously reported rust genes identified polymorphic SSR markers on LG 13. So far, R_{TX16R} was mapped between ORS316 and HT382 on LG 13, with seven linked markers covering a distance of 31.6 cM. The closest marker is ORS316, at 5.1 cM. The closely linked markers to the two resistance genes will facilitate marker-assisted selection (MAS) and gene pyramiding, and also provide a basis for further characterizing and cloning of the two genes.

Introduction

Sunflower, the fifth largest oilseed crop in the world, plays an important role in human diets. Downy mildew, caused by the fungus *Plasmopara halstedii* (Farl.) Berl. & de Toni, is one of the more destructive diseases of cultivated sunflower (*Helianthus annuus* L.). Sunflower production in North America also has suffered serious yield losses from newly evolved races of sunflower rust (*Puccinia helianthi* Schwein.). At least 15 downy mildew resistance (PI) genes have been mapped, on LGs 1 (PI_{Arg} , PI_{13} , PI_{14} , and PI_{16}), 2 (PI_{18}), 4 (PI_{17}), 8 (PI_1 , PI_2 , PI_6 , PI_7 , and PI_{15}), and 13 (PI_5 , PI_8 , PI_{42B} , and PI_{803}), respectively (reviewed by Liu et al. 2012; Qi et al. 2015b, c). Ten rust resistance genes (R -genes) have been mapped on the sunflower SSR map, with LG 13 containing most of the genes (R_4 , R_{11} , R_{adv} , R_{13a} (R_{HAR6}), and R_{13b}), and the other five genes located on LGs 2 (R_5), 8 (R_1), 11 (R_{12} and R_{14}), and 14 (R_2) (Gong et al. 2013a, b; Qi et al. 2011b; 2015a; reviewed by Zhang et al. 2015, unpublished). Molecular mapping of the two resistance genes in TX16R will provide useful markers for marker-assisted breeding for both sunflower downy mildew and rust resistance.

Objectives

1. Analyze the relationship of the PI genes in seven germplasms.
2. Molecularly map the downy mildew resistance gene PI_{TX16R} and the rust resistance gene R_{TX16R} from TX16R.

Materials and Methods

1. **Half-diallel test:** 21 F_2 progeny populations of seven germplasms were tested for resistance segregation to downy mildew 'hot' race 734 in 2011 and 2015, including HAR 4, HAR 5, TX16R, RHA 419, RHA 340, HA 458, and RHA 428.
2. **Mapping population:** F_2 population of 153 and 155 individuals derived from HA 434 x TX16R was used to map PI_{TX16R} and R_{TX16R} , respectively.
3. **Assessment of the F_2 individuals for downy mildew and rust resistance:** The germination, inoculation and sporulation for downy mildew race 734 followed Mulpuri et al. (2009) in 2011. Inoculation for rust races 777 and 336 followed the method of Qi et al. (2011a, b) in 2011 and 2012, respectively.
4. **Polymorphism screening:** Bulk segregant analyses were conducted using SSR and EST-SSR primers from LG 4 for mapping of PI_{TX16R} , and LGs 2, 11, and 13 for mapping of R_{TX16R} . Polymorphism screening between parents: SSR and EST-SSR markers mapped to LGs 4 and 13.
5. **Statistical analysis and linkage map construction:** Chi-square test and the MAPMAKER/Exp version 3.0b program (Lander et al. 1987).

Results and Discussion

1. Segregation of downy mildew resistance F_2 progenies of half-diallel crosses among seven germplasms suggested that PI_{TX16R} is localized on LG 4, the same as PI_{17} from HA 458, with no recombinant detected. PI_{42B} in RHA 428 is tightly linked with PI_8 in RHA 340, with one recombinant observed in 490 F_2 progenies (Table 1).

Table 1. Segregation of downy mildew resistance in 21 F_2 populations derived from half-diallel crosses (Susceptible/Resistant progenies).

Parents	HAR 4	HAR 5	TX16R	RHA 419	RHA 340	HA 458
HAR 5	0/222					
TX 16	12/102	13/194				
RHA 419	16/130	6/93	11/114			
RHA 340	3/87	12/135	9/100	12/145		
HA 458	4/172	3/99	0/339	5/38	11/128	
RHA 428	7/132	6/93	9/137	14/127	1/489	9/124

2. Resistance segregation for downy mildew and rust in the mapping population confirming a single dominant gene controlling the resistance to downy mildew and rust in TX16R, respectively (Table 2).

Table 2. Segregation of the downy mildew resistance locus PI_{TX16R} , the rust resistance locus R_{TX16R} , and the SSR (ORS), and EST-SSR (HT) markers linked to them in the F_2 population of the cross HA 434 x TX16R.

Traits or markers	Number of F_2 plants	Observed number ^a				Ratio tested	χ^2	P
		A	H	B	D			
PI_{TX16R}	153	30	82	41		1:2:1	2.37	0.305
ORS644	152	30	82	40		1:2:1	2.26	0.323
ORS963	151	29	82	40		1:2:1	2.72	0.256
ORS620	149	28	79	42		1:2:1	3.17	0.204
ORS161	153	30	79	44		1:2:1	2.73	0.256
ORS523	152	30	78	44		1:2:1	2.68	0.261
ORS695	152	30	78	44		1:2:1	2.68	0.261
ORS1239	152	29	78	45		1:2:1	3.47	0.176
R_{TX16R}	155	39	81	35		1:2:1	0.52	0.770
ORS224	155	42	78	35		1:2:1	0.64	0.727
ORS1179	155	39	81	35		1:2:1	0.52	0.770
ORS995	155	40	77	38		1:2:1	0.06	0.971
HT1040	155	41	76	38		1:2:1	0.17	0.917
ORS581	155			36	119	3:1	0.26	0.610
ORS316	155	40	78	37		1:2:1	0.12	0.941
HT382	155	34	75	46		1:2:1	2.02	0.364

a: Symbols: A, HA 434 (p/pl , rr); H, heterozygous (P/pl , R/r); B, TX16R (PI/PI , RR); D, Rr or rr .

3. Molecular mapping localizing the PI_{TX16R} gene on LG 4, with a marker ORS963 co-segregating, and ORS644 tightly linked to PI_{TX16R} at a genetic distance of 0.3 cM (Figure 1).
4. Molecular mapping localizing the R_{TX16R} gene on LG 13, between the markers ORS316 and HT382 (Figure 1). The closest marker so far is ORS316, at a genetic distance of 5.1 cM. Comparison of this rust resistance gene with other mapped R -genes suggests that R_{TX16R} may belong to R -gene sub-cluster II on LG 13 (Gong et al. 2013b).

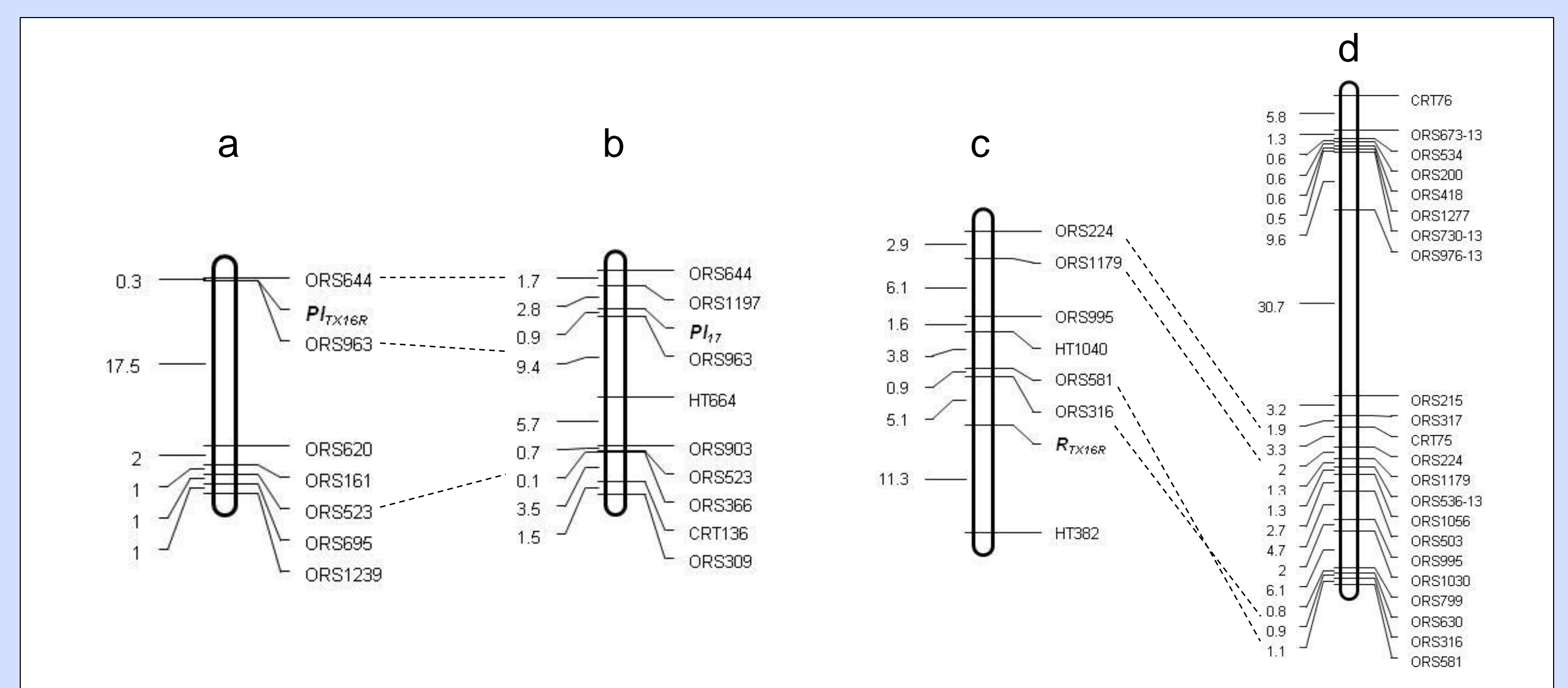


Figure 1. a) Mapping result of the PI_{TX16R} gene on LG 4, with seven linked SSR (ORS) markers, based on the analysis of 153 F_2 plants derived from the cross of HA 434 x TX16R. b) Modified mapping result of PI_{17} on LG 4 (Qi et al. 2015c). c) Mapping result of the R_{TX16R} locus on LG 13, with five linked SSR and two EST-SSR (HT) markers, based on the analysis of 155 F_2 plants. d) A simplified reference map of LG 13 of the map composite_Tang 2003b in the Sunflower CMap Database (http://sunflower.uga.edu/cgi-bin/cmap/map_search). The distances are given in centimorgans (cM). The common markers are aligned between maps.

Future work

1. Additional markers, such as SNPs, resistance gene candidate (RGC) markers, and newly designed SSR markers are required to fine map PI_{TX16R} and R_{TX16R} .
2. Further characterization will be needed to analyze the relationships between PI_{TX16R} and PI_{17} and R_{TX16R} and other rust resistance genes on LG 13.
3. The resistant progenies derived from TX16R will be further screened, which may be used as a differential line to identify different downy mildew and rust races in the future.

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