Genome localization of rust resistance genes

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ABSTRACT

- Sunflower rust, caused by *Puccinia helianthi*, is common and widespread all over the main sunflower production areas of the world. The rapid changes that occur in the virulence of this pathogen are a continuous threat to the effectiveness of existing rust-resistant inbred lines and hybrids. Several sources conferring resistance to rust have been identified in sunflower, but only a few of them have been genetically characterized, mapped and linked to molecular markers. The objectives of this work were to determine the genetic control of rust resistance in previously uncharacterized sources and to map the factors involved in this response.
- Six mapping populations were obtained using the resistance sources P386, HAR4, HAR6, Caburé Precoz, B648, and PNR1, and the susceptible line R702. F_3 families derived from single F_2 plants by selfing were evaluated by their resistance to rust isolate B.A.&S. 2009 (race 760) which discriminates the resistant sources from the susceptible line. At least 200 SSR markers were used for polymorphism detection among resistant and susceptible parents, and a bulk segregant analysis (BSA) strategy was used in order to identify genomic regions involved in resistance.
- Evaluation of the $F_{2:3}$ families of each population indicated a monogenic control of resistance for each of the sources. Thirty eight to sixty one percent of the assayed SSRs were polymorphic in each population. BSA analyses showed cosegregation among markers located on linkage group (LG) 13 and the factors controlling resistance in each of the analyzed rust resistance sources.
- LG13 was previously described as a genomic region that harbors rust resistant genes from other sources. The six sources analyzed in this work might carry new alleles of the already known genes of this region, or new genes that are closely linked to them. Fine mapping and allelism tests using these genetic factors are being carried out to decipher both alternatives.
- LG13 is a genomic region populated of resistant gene analogs and disease resistant genes. Fine mapping of the rust resistant genetic factors on this region, elimination of linkage drag from wild sources, and gene pyramidization is a reliable way to achieve a sustainable genetic control of sunflower rust.

Key words: bulk segregant analysis, mapping, molecular breeding, *Puccinia helianthi,* sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus*) is an oilseed crop highly cultivated in many countries, being mainly used for edible oil production. During the XVI century, sunflower was introduced into European countries where suffered its transformation to a commercial crop. Sunflower is usually growth in template-warm regions of the world where it is exposed to a high diversity of pathogens.

Puccinia helianthi, the most common rust on cultivated and wild sunflower, is found in every sunflower-growing country and has caused serious yield losses in Argentina, Canada, Russia, USA, and Australia. Replacement of susceptible open-pollinated cultivars with hybrids possessing resistance to one or more races has greatly decreased losses due to rust. However, as the rust population changes and races able to attack resistant hybrids increase in frequency, rust epidemics have reoccurred. On oilseed hybrids, rust causes yield losses primarily by reducing head diameter and seed size, and by causing a reduction in oil content (Siddiqui and Brown, 1977; Zimmer and Zimmerman, 1972).

The existence of physiological races of *P. helianthi* was suspected early in the XX century (Bailey, 1923), but Sackston (1962) was the first to identify them. Using three lines developed by the Canadian Department of Agriculture (Putt and Sackston, 1957), four races were identified on cultivated sunflower in Canada, which were designated as North American (NA) Races 1, 2, 3, and 4.

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MATERIALS AND METHODS

Six mapping populations were obtained using the resistance sources P386, HAR4, HAR6, Caburé Precoz, B648, and PNR1, and the susceptible line R702. P386 is a rust resistance inbred line of Argentinean origin which was reported by Yang (1989) to be resistant to NA races 1, 2, 3 and 4. This resistance is conferred by a single genetic factor that was named *Pu6*, which is not allelic to the previously reported genes R_1, R_2, R_4 y R_5. The inbred line HAR4 is also resistant to NA race 4 and it was derived from Saenz Peña 74-1-2, another Argentinean sunflower OPP (Miller et al., 1988). The gene responsible for this resistance was proposed to be allelic to the factors carried by HAR1, HAR3 and HAR5. Caburé Precoz, is an OPP derived from the Argentinean population Charata, which was also described as resistant. B648 is a Nidera proprietary maintainer inbred line and PNR1 is a line derived from a commercial hybrid from the company Pannar. All of these sources are resistant to isolate B.A.&S. 2009 belonging to race 760.

At least 100 $F_{2:3}$ families derived from single F_2 plants by selfing were evaluated by their resistance to rust isolate B.A.&S. 2009. Twenty five seeds of each of the 100 $F_{2:3}$ families of each population were planted in 20x20x30cm pots, given a total of approximately 2500 F_3 individuals per each population. Twenty seeds of each of the parental lines were planted in three replications and used as controls. Plants were grown in a greenhouse under natural light conditions supplemented with 400 W halide lamps to provide a 16 h day length. Day/night temperatures were 25 and 20ºC, respectively.

To conduct phenotypic analysis of rust resistance, a total of 100 $F_{2:3}$ families, along with the parental lines and 10 F_1 plants of each population were inoculated with *P. helianthi* spores of race 760 at the V2 stage of development (Schneiter and Miller, 1981), using the procedure described by Gulya (1996). The evaluation of symptoms were conducted twelve days after inoculation, using a 0-4 rating that classifies 0,1, and 2, as resistant plants, and 3 and 4, as susceptible (Yang et al., 1986). In the case of $F_{2:3}$ families, each plant was individually scored and the proportion of susceptible plants were obtained; thus, $F_{2:3}$ families with 100 and 0% proportions were scored as susceptible and resistant respectively, while the remaining proportions were considered segregating families.

Leaf tissue of individuals F_2 plants of each population and parental lines were collected and DNA were extracted using the Qiagen DNeasy 96 Plant Kit (Qiagen Inc., USA). At least 200 SSR markers were used for polymorphism detection among resistant and susceptible parents, and a bulk segregant analysis (BSA) strategy (Michelmore et al., 1991) was used in order to identify genomic regions involved in resistance. To do that, markers that showed polymorphism between parental lines were used to analyze 6 DNA bulks, 3 of them composed by DNA of 7 resistant F_2 plants each and the other 3 composed by DNA of 7 susceptible F_2 plants each. The PCR assays were conducted in 10µl reaction volume containing

1x PCR buffer, 400µM dNTPs, 2.5 mM $MgCl_2$, 0.5 U Taq DNA Polymerase (Biotools, Madrid, Spain), 0,4 µM of each primer and 50ng of genomic DNA. PCR cycling conditions were as follows: an initial denaturation step at 95°C for 3 min, followed by 38 cycles of 94°C for 30s, 50°C for 30s, 72°C for 45s, and a final extension step at 72°C for 10 min.

The PCR products were visualized on either 4% Metaphor agarose stained with SybrSafe (Invitrogen Life Technologies, Carlsbad, USA), or 6% polyacrylamide gels with nitrate staining (Silver Sequence; Promega Biotech, Madison, USA).

RESULTS

R702 showed severe disease symptoms, with big pustules, after being inoculated with rust isolate B.A.&S. 2009. All the resistant parents and the F_1 plants sown together showed no infection (Table 1). Observed and expected ratios of resistant and susceptible progenies in each of the six crosses conform to a single locus model of inheritance. Factors controlling resistance in each population behave as dominant taken into account the results obtained in $F_{2:3}$ population analysis and in the F_1 hybrids obtained from resistant by susceptible crosses (Table 1).

Table 1. Observed segregation of Resistant (R), Segregant (Seg) and Susceptible (S) in F_1 and $F_{2:3}$ families to rust isolate B.A.&S. 2009 (race 760). Chi-square tests of single locus model for control of resistance are also showed.

	F1		F2:3				
	Number of plants		Number of families			Ratio tested	χ^2 p value
Cross	R	S	R	Seg	S		
HA-R4/R702	10	0	30	51	28	1:2:1	0.77
HA-R6/R702	10	0	26	48	22	1:2:1	0.84
P386/R702	10	0	25	53	22	1:2:1	0.65
Cabure Precoz/R702	10	0	34	58	28	1:2:1	0.73
B648/R702	10	0	23	50	20	1:2:1	0.72
PNR1/R702	10	0	32	60	35	1:2:1	0.77

Bulk segregant analysis conducted over each population, permits to identify cosegregation among two markers located on linkage group (LG) 13, ORS316 and ORS317, and the factors controlling rust resistance in each of the analyzed population. Taking into account these results, three additional markers of LG13 were used to detect polymorphism: HRG01 (Horn el at., 2003), ZVG61 and ORS581 (Table 2).

Table 2. Bulk segregant analysis of six different mapping populations, using five markers located on LG13.

		Molecular marker				
Population	**Bulk**	**ORS317**	**HRG01**	**ZVG61**	**ORS581**	**ORS316**
|HA-R6/R702	**R1**	-	a[1]	a	a	a
	R2	-	a/b	a	a	a
	R3	-	a	a	a	a
	S1	-	b	b	b	b
	S2	-	a/b	a/b	b	a/b
	S3	-	a/b	b	b	b
Cabure/R702	**R1**	a	a/b	-	-	a
	R2	a/b	a	-	-	a
	R3	a	a/b	-	-	a
	S1	b	b	-	-	b
	S2	a/b	a/b	-	-	b
	S3	b	a/b	-	-	b
P386/R702	**R1**	a/b	a/b	-	-	a
	R2	a/b	a	-	-	a
	R3	a	a/b	-	-	a
	S1	b	b	-	-	b

	S2	a/b	a/b	-	-	b
	S3	b	a/b	-	-	b
HAR4/R702	**R1**	a	-	a	-	a
	R2	a/b	-	a/b	-	a
	R3	a	-	a	-	a
	S1	b	-	b	-	b
	S2	a/b	-	b	-	a/b
	S3	b	-	b	-	b
B648/R702	**R1**	a	a	-	-	-
	R2	a	a	-	-	-
	R3	a	a	-	-	-
	S1	b	b	-	-	-
	S2	a/b	b	-	-	-
	S3	b	b	-	-	-
PNR1/R702	**R1**	a	-	-	-	a
	R2	a	-	-	-	a/b
	R3	a	-	-	-	a
	S1	b	-	-	-	b
	S2	a/b	-	-	-	b
	S3	b	-	-	-	b

[1] Alleles 'a' and 'b' did not represent the same molecular weight for each population. Allele a (grey shaded) always represents the allele found in the resistant parental line, while b represents the allele fund in the susceptible parental line.

DISCUSSION

Inheritance of rust resistance was reported previously for two out of the six sources used in this work. Pu_6 controls the resistance in P386 and R_4 is the resistant factor present in HAR4. Inheritance of rust resistance of the remaining four sources is reported here for the first time.

The obtained results indicate that each of the analyzed rust resistance sources possess a single dominant factor controlling resistance located in LG13. At least for HAR4 this is not a surprising result. Miller et al. (1988) showed that this line carries a resistant factor which is allelic to a resistance gene founded in HAR3 and this factor was mapped on LG13 (Qi et al., 2011). The resistance gene Pu_6 from P386 was genetically characterized by Yang el at. (1986) and they demonstrated that this factor is not allelic to R_4. For this reason it is interesting that our results showed that Pu_6 is located in the same LG as R_4.

In fact, LG13 was described previously as a region in the sunflower genome that harbors several rust resistant factors. R_{Adv}, from a proprietary Australian hybrid, was the first rust resistance gene mapped on LG13 (Lawson et al., 1998; Yu et al., 2003). Later R_{Adv} from the USDA line Rha340 (Bachlava et al. 2009) and R_4 from HAR3 (Qi et al., 2011) were also localized in LG13.

P386, HAR4, and the other four sources analyzed in this work might carry new alleles of the already known genes of this region, or new genes that are closely linked to them. Fine mapping and allelism tests using these genetic factors are being carried out to decipher both alternatives.

LG13 is a genomic region populated of resistant gene analogs and disease resistant genes. Fine mapping of the rust resistant genetic factors on this region, elimination of linkage drag from wild sources, and gene pyramidization is a reliable way to achieve a sustainable genetic control of sunflower rust.

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