Pathological and morphological evaluation of sunflower isohybrids carrying or not the *Rcm-1* gene for *Sunflower chlorotic mottle virus* resistance

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ABSTRACT

Sunflower (*Helianthus annuus L.*) crops are affected by *Sunflower chlorotic mottle virus* (SuCMoV) which reduces yield parameters in commercial hybrids infected at early ontogenic stages. Sunflower isohybrids (between two near isogenic males differing in the *Rcm-1* resistance gene and three different females) were mechanically inoculated with SuCMoV under field conditions in two locations (Balcarce and Venado Tuerto) and evaluated for symptom expression and agronomic characteristics. Symptoms were scarce chlorotic pinpoint in all resistant hybrid combinations and severe chlorotic mottling in all susceptible hybrids, independently of the female parents. Nevertheless, higher symptoms intensity was detected in Venado Tuerto. Morphological parameters were more affected in Venado Tuerto than in Balcarce and differed among hybrid combinations.

Key words: *Helianthus annuus* – isohybrids – *sunflower chlorotic mottle virus* – virus.

INTRODUCTION

Sunflower chlorotic mottle virus (SuCMoV) is a potyvirus, which seemed to be restricted to the Americas. In Argentina, it has been associated with chlorotic mottling and plant stunting symptoms and it has been reported in several provinces including; Entre Ríos, Santa Fé, Buenos Aires and Córdoba. The virus is a member of the *Potyvirus* genus within the Potyviridae family (Dujovny et al., 1998, 2000) and it has been classified as a strain of *Potato virus Y* (Berger, 2005).

Recently, a sunflower line tolerant to SuCMoV infection has been reported (L33) and the resistance gene *Rcm-1*gene has been mapped (Lenardon et al., 2005). Breeding for virus resistance is one of the best ways to manage virus epidemics since no additional agricultural practices are required to reduce disease incidence and severity. Using molecular marker-assisted selection, the *Rcm-1*gene was incorporated to a susceptible L37S male in order to obtain a near-isogenic resistant version L37R. The objective of this work was to study the level of resistance obtained by the presence of this gene in different hybrid combinations, both in terms of symptom expression and agronomic characteristics.

MATERIALS AND METHODS

Plant Material and Experimental design

Crosses between a pair of near-isogenic male lines with (R) and without (S) the *Rcmo-1* gene and 3 susceptible female lines were performed in order to obtain 3 pairs of isohybrids. Hybrids obtained from the cross between the three females and the resistant donor L33 (source of *Rcm-1* gene) (Advanta Semillas S.A.I.C) were employed as controls.

Split plot design experiments with three replications were sown in two locations: Venado Tuerto (Santa Fe Province, 33º45´S, 61º 58´ W, November 20, 2006) and Balcarce (Buenos Aires Province, 37° 45' S, 58° 18' W, November 27, 2007). Each replication consisted of the hybrids as the main plot, and two treatments (SuCMoV inoculated versus non-inoculated) as the subplot. Each subplot was represented by three rows (20 plants per row). The middle row was used for treatment application and evaluation.

Plant inoculations
A SuCMoV isolate maintained on sunflower commercial hybrid CF 7 under greenhouse conditions was used as inoculum source for the whole experiment. Sunflower plants were mechanically inoculated at vegetative stage V 12 (Venado Tuerto) and at R1 (Balcarce) (Schneiter and Miller, 1981) with a high pressure airbrush apparatus, using a slurry prepared from infected leaves ground with phosphate buffer and abrasive (Lenardon et al., 2005).

Evaluation
Inoculation in Venado Tuerto and Balcarce were performed on December 20, 2006 and on January 15, 2007 respectively. . Symptom expression was evaluated 25 days after the inoculation and agronomic parameters (flowering date, plant height, capitulum diameter, length and width of a totally expanded leaf from middle portion of the stem) at proper times.

Statistical analysis
Results were analyzed by ANOVA. Orthogonal contrasts were planned in order to test the effects on the morphological characters of: a) the treatment (inoculated vs non-inoculated) for each hybrid and 2) the effect of the incorporated gene (R vs S) within each treatment (inoculated or non- inoculated).

RESULTS AND DISCUSSION

Inoculation was successful and all inoculated plants expressed virus symptoms. A heavy storm occurred in Venado Tuerto and some plants suffered mechanical stress and lodging before flowering so one pair of isohybrids was eliminated from the analysis.

Qualitative differences between symptoms in all isohybrid pairs were detected as expected. In all resistant hybrids symptoms were scarce chlorotic pinpoints, similar to those observed in the controls when the resistant L33 donor was employed. Nevertheless, the intensity of the symptoms was higher in Venado Tuerto than in Balcarce even in the crosses where L33 was a parent. In the first location, the chlorotic pinpoint was intense and also chlorotic ringpots were detected. In Balcarce, typical scarce chlorotic pinpoint symptoms (SCP) were observed on resistant genotypes. The susceptible counterpart of the isohybrids exhibited severe chlorotic mottling (SCM) symptoms independently of hybrid combination (Table 1).

Table 1. Symptom expression in isohybrid pairs differing in *Rcm-1* gene artificially inoculated in two locations[1].

Pedigree	Venado Tuerto	Balcarce
L16xL37S	SCM	SCM
L16xL37R	ICP + CR	SCP
L16xL33	ACP + CR	ACP + CR
L348xL37S	SCM	SCM
L348xL37R	ICP + CR	SCP
L348xL33	ACP + CR	SCP
L351xL37S	NR	SCM
L351xL37R	NR	SCP
L351xL33	NR	SCP

[1]SCM: severe chlorotic mottle; SCP: scarce chlorotic pinpoints; ACP: intense chlorotic pinpoint; ICP: intermediate chlorotic pinpoint; CR: chlorotic ringspots; NR: no results

One-two day differences in flowering date between treatments were observed according to the isohybrid (data not shown).

ANOVA analysis for both locations detected significant differences between hybrids and treatments and an interaction between hybrid x treatment ($P<0.05$) for the morphological characters with exception of head diameter in Balcarce. The means of morphological traits are presented in Table 2.

Table 2. Morphological traits in isohybrid pairs differing in the *Rcm-1* gene inoculated and non-inoculated with SuCMoV in two locations. Hybrids with L33 (resistant source) are used as controls.

Venado Tuerto

Pedigree	Plant height, cm		Head diameter, cm		Leaf length, cm		Leaf width, cm	
	Inoc.	Non inoc.	Inoc.	Non inoc.	Inoc.	Non inoc.	Inoc.	Non inoc.
L16xL37S	171	193	9	21	17	23	17	23
L16xL37R	196	203	11	18	21	24	21	24
L16xL33	177	181	13	17	23	25	21	23
L348xL37S	179	216	8	18	17	23	16	22
L348xL37R	217	222	15	19	22	23	22	23
L348xL33	195	200	12	18	19	24	17	22

Balcarce

Pedigree	Plant height, cm		Head diameter, cm		Leaf length, cm		Leaf width, cm	
	Inoc.	Non inoc.	Inoc.	Non inoc.	Inoc.	Non inoc.	Inoc.	Non inoc.
L16xL37S	173	186	12	11	20	25	19	23
L16xL37R	185	190	12	13	25	24	25	22
L16xL33	156	152	17	16	30	31	30	31
L348xL37S	180	187	12	14	17	25	15	24
L348xL37R	191	193	13	13	22	24	20	23
L348xL33	154	160	19	20	26	28	27	28
L351xL37S	171	172	17	16	20	25	19	23
L351xL37R	172	176	17	16	26	27	25	27
L351xL33	152	152	22	20	27	26	28	26

Table 3. Orthogonal contrast for morphological traits between inoculated and non-inoculated hybrids, which differ in *Rcm-1* gene in two locations.

Venado Tuerto

Inoculated vs. non-inoc.	Plant height			**Leaf length**			**Leaf width**			**Head diameter**		
	MS	F Value	p-value	MS	F Value	p-value	MS	F Value	p-value	MS	F Value	p-value
L16 xL37S	506.25	16.99	0.0026	62.08	72.40	<0.0001	65.34	80.47	<0.0001	108.37	65.49	<0.0001
L16xL37R	73.50	2.47	0.1507	16.67	19.44	0.0009	17.68	21.78	0.0005	83.10	50.22	0.0001
L348 xL37S	2041.31	68.51	<0.0001	54.00	62.97	<0.0001	51.04	62.86	<0.0001	155.14	93.75	<0.0001
L348xL37R.	48.17	1.62	0.2354	0.74	0.86	0.3728	2.04	2.51	0.1388	28.17	17.02	0.0026

Balcarce

Inoculated vs. non-inoc.	Plant height			**Leaf length**			**Leaf width**		
	MS	F Value	p-value	MS	F Value	p-value	MS	F	p-value
L16 xL37S	261.36	20.25	0.0003	36.51	9.79	0.0058	20.91	3.94	0.0626
L16 xL37R	44.61	3.46	0.0794	5.61	1.50	0.2359	9.63	1.81	0.1946
L348 xL37S	84.68	6.56	0.0196	96.0	25.75	0.0001	105.84	19.95	0.0003
L348 xL37R	10.32	0.80	0.3829	6.83	1.83	0.1927	8.64	1.63	0.2181
L351 xL37S	7.28	0.56	0.4623	26.46	7.10	0.0158	25.63	4.83	0.0413
L351 xL37R	2.04	0.16	0.6955	1.50	0.40	0.5339	5.61	1.06	0.3175

As previously described, symptoms intensity was higher in Venado Tuerto than in Balcarce. Thus, the inoculated plants in Venado Tuerto showed a significant reduction in all parameters in the S hybrids (without resistant gene) and in the R hybrids (with resistant gene) except plant height of R hybrids and leaf size (when L348 female was crossed with L37 R) (Table 3). On the contrary, in Balcarce, inoculation showed a significant reduction in all parameters in the S hybrids but it did not affect R hybrids (Table 3).

The presence of the incorporated gene did not modify the morphological characteristic of non-inoculated isohybrids (S vs R non-inoculated) (Table 4).

Table 4. Orthogonal contrast for morphological traits, between hybrids differing in *Rcm-1* gene inoculated and non-inoculated with SuCMoV in two locations.

Venado Tuerto

Pedigree	Plant height			**Leaf length**			**Leaf width**			**Head diameter**		
	MS	F Value	p-value	MS	F Value	p-value	MS	F Value	p-value	MS	F Value	p-value
L16 xL37S vs L16xL37R.inoc	684.7	23.0	0.001	24.8	28.9	0.0002	22.8	28.1	0.0002	0.2	0.1	0.7553
L16 xL37S vs L16xL37R.non inoc	170.7	5.7	0.040	1.4	1.6	0.225	0.8	1.0	0.339	9.8	5.9	0.0379
L348 xL37S vs L348xL37R. inoc	2140.6	71.8	<0.0001	40.0	46.7	<0.0001	49.3	60.7	<0.0001	61.3	37.0	0.0002
L348 xL37S vs L348xL37R. non inoc	64.4	2.2	0.176	0.03	0.03	0.863	1.7	2.1	0.173	0.5	0.3	0.6111

Balcarce

Pedigree	**Plant height**			**Leaf length**			**Leaf width**		
	MS	F Value	p-value	MS	F Value	p-value	MS	F Value	p-value
L16 xL37S vs L16xL37R.inoc	234	18.1	0.0005	36.5	9.8	0.006	39.5	7.5	0.0137
L16 xL37S vs L16xL37R.non inoc	33.8	2.6	0.1232	5.6	1.5	0.236	1.9	0.4	0.5542
L348 xL37S vs L348xL37R. inoc	200.3	15.5	0.0010	29.0	7.8	0.012	36.5	6.9	0.0172
L348 xL37S vs L348xL37R. non inoc	66.7	5.6	0.0355	3.2	0.9	0.365	1.7	0.3	0.5776
L351 xL37S vs L351xL37R. inoc	2.1	0.2	0.6889	55.2	14.9	0.001	43.7	8.3	0.0101
L351 xL37S vs L351xL37R. non inoc.	0.04	0.003	0.9580	12.3	3.3	0.086	15.4	2.9	0.1060

Complete resistance to SuCMoV has not been detected up to now. The *Rcm-1* gene produced a qualitative modification of symptom expression, which could be affected by the environment and the specific hybrid combination. Slight differences in the inoculation time could be excluded as the cause of the intensity differences between locations, because the phenotypic data used for gene mapping was obtained under field inoculation in Balcarce at younger stages and the symptoms of resistant plants were equal to those obtained for this location in the present study (Lenardon et al., 2005).

The use of this resistance gene could attenuate the effect of SuCMoV on morphological traits such as leaf area, and therefore, on some of the yield components.

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