Resistance to *Tomato leaf curl New Delhi virus* Derived from WM-7: Genetic Control and Introgression into Traditional Melon Backgrounds

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Introduction

Melon (*Cucumis melo* L.) is one of the most important crops of the *Cucurbitaceae* family. The world production of this vegetable was more than 27 million tons in 2019 (FAOSTAT, 2021). Spain is one of the ten countries with the highest production and the first producer and exporter in the European Union. The most important melon types cultivated in Spain belong to the *ibericus* group, which includes 'Piel de Sapo', 'Amarillo', 'Tendral', 'Rochet' and 'Blanco' melons. Landraces of snake melon (*flexuosus* group), highly appreciated in other countries, are also cultivated in some eastern coastal regions of Spain, under the name of 'Alficoz'.

Currently one of the major production constraints of cucurbits is the increase in viral diseases due to globalization and climate change, which facilitate their expansion. One of these viral diseases is caused by the whitefly-transmitted geminivirus *Tomato leaf curl New Delhi virus* (ToLCNDV). Plants affected with this disease show vein clearing, yellow mottling, crinkling, puckering, and upward or downward curling of leaves, besides sterility and poor fruit setting (Jyothsna et al. 2013). The first detection of ToLCNDV was in 1995 on tomato (*Solanum lycopersicum* L.) in India (Srivastava et al. 1995). Soon, the host range and the expansion area were extended. The first report of ToLCNDV in Spain was in 2012 in zucchini (*Cucurbita pepo* L.) in Murcia (Juárez et al. 2014).

The development of resistant varieties is one of the best long-term safe and sustainable approaches to manage viral diseases. In previous works of the research group, López et al. (2015) identified resistance to ToLCNDV in five melon genotypes from India; three of them belonged to the *momordica* group and two were wild *agrestis.* Resistance derived from the wild *agrestis* WM-7 has been reported to be controlled by one major dominant *locus* in chromosome 11 and two additional regions in chromosomes 2 and 12 (Sáez et al. 2017). The breeding program for the introgression of this resistance into traditional sweet melon and snake melon genetic backgrounds has been initiated.

The aim of this work was to further study the genetic control of the resistance derived from WM-7, by fine mapping the major *locus* on chromosome 11 and by analyzing the effect of the chromosome 12 region on the resistance. The plant materials developed, and the molecular markers identified will be useful in the breeding program for the introgression of resistance to ToLCNDV in different traditional genetic backgrounds.

Material and Methods

Plant material: The resistance source WM-7 was crossed with seven homozygous lines derived from melon landraces: two 'Piel de Sapo' (11PS-BGV013188 and 03PS-BGV016356), two 'Blanco' (29BL-BGV015753 and 32BL-BGV016453), one 'Amarillo' (22AM-GO-BGV016451), one 'Rochet' (02RC-BGV003718) and one 'Alficoz' (05AL-BGV004853). These F_1 generations were backcrossed to each of the landraces to obtain the backcross generations (BC_1), except for the F_1 with 02RC, which was selfed to produce the F_2 generation (Table 1).

Between 20 and 40 plants of each generation (Table 1) were evaluated for resistance to ToLCNDV. The rest of the BC_1 and F_2 plants (50 per genotype) were used to progress in the introgression of the resistance into the traditional genetic backgrounds. These latter plants were genotyped with a set of markers located on the candidate regions of the resistance to ToLCNDV. Selected genotypes were selfed to obtain the corresponding either BC_1S_1 or F_3 generations. These generations were evaluated for resistance to ToLCNDV.

Inoculation and disease assessment: Inoculation was carried out as described in Lopez et al. (2015). In brief, a ToLCNDV-infectious clone was agroinoculated by injection into petioles of MU-CU-16 zucchini plants. Fifteen days later, leaf tissue from the symptomatic MU-CU-16 plants was mashed with inoculation buffer and used to mechanically inoculate the melon plants, at the two true-leaf stage. One cotyledon and the bigger true-leaf were dusted with carborundum 600 mesh and then were rubbed with a cotton-swab impregnated with the homogenate inoculum. All the plants were reinoculated 10 days later.

Disease assessment was carried out by visual scoring of symptom at 15- and 30-days post inoculation (dpi), following the scale described in López et al. (2015), which ranged from 0 (asymptomatic plants) to 4 (severe symptoms). Plants with no or mild symptoms (0 to 1 in the scale) were considered resistant and plants with moderate to severe symptoms (2 to 4 in the scale) were considered susceptible. Quantitative PCR was carried out as previously described (Sáez et al. 2017) to quantify the amount of virus in DNA isolated from the apical leaves using the CTAB method (Doyle and Doyle 1990).

SNP Genotyping: Fifty plants of each of the BC_1 and F_2 plants used in the backcrossing program were genotyped with a panel of 23 SNPs, covering the three genomic regions associated with resistance in chromosomes 2, 11, and 12 (Sáez et al. 2017). DNA was isolated using the CTAB method (Doyle and Doyle, 1990) and the genotyping was done using the Agena Bioscience platform ('Epigenetic and Genotyping unit of the University of Valencia, Unitat Central d´Investigació en Medicina (UCIM), Spain').

Results and Discussion

Six BC_1 and one F_2 generations derived from the resistance source WM-7 in different traditional genetic backgrounds were evaluated for resistance to ToLCNDV. Segregation was observed in all progenies (Table 1). Plants asymptomatic or showing slight symptoms, with lower virus titer, were classified as resistant, while those displaying moderate to severe symptoms and with high viral accumulation were considered susceptible (Figure 1). Segregation fitted the expected ratio of resistant and susceptible plants for a single dominant gene, except for two BC_1 generations. These two BC_1 progenies had an excess of resistant plants, which could correspond to escapes or to late infections. Sáez et al. (2017) also obtained segregations compatible with a monogenic dominant model in F_2 and BC generations derived from the cross WM-7 x Piñonet Piel de Sapo.

Fifty plants of each of the BC_1 and F_2 generations were genotyped with the ToLCNDV set of SNPs, which included markers in the candidate regions in chromosomes 2, 11 and 12. A selection of certain genotypes was carried out to obtain the selfing progenies (Table 2). Recombinants in chromosome 11 were selected (F_3 derived from 02RC genetic background, and BC_1S_1 derived from 05AL genetic background), with the purpose of narrowing the candidate interval (Table 2). Besides that, plants that only included the candidate region in chromosome 12 in heterozygote state were chosen (BC_1S_1 derived from the genetic backgrounds of 29BL, 32BL, 22AM-GO and 05AL), in order to better analyze the effect of this region on resistance (Table 2).

One of the F_2 plants derived from 02RC genetic background was homozygous for the WM-7 alleles in the candidate region of chromosome 11 between markers SNPCmND7 and SNPCmND16bis and heterozygous below this marker (Table 2). All the selfing descendants of this plant remained asymptomatic when inoculated with ToLCNDV. Virus titer in these plants was also significantly lower than that detected in susceptible plants at 15 and 30 dpi. These results allowed delimiting the candidate region over SNP SNPCmND17 (at position 30,410,537 bp). The selfing progeny from the other F_2 from 02RC, which was heterozygote between markers SNPCmND7 and SNPCmND13bis and homozygous for the allele of 02RC below this marker, segregated for resistance, thus allowing the narrowing of the candidate region, over marker SNPCmND15 (at position 30,377,414). The BC_1 plant derived from 05AL did not carry this region, so the segregation for resistance observed in BC_1S_1 progeny must be explained by the region of chromosome 12 or other regions of the genome. The candidate region proposed here would then expand between markers SNPCmND7 and SNPCmND15 (positions 30,249,798-30,377,414 bp), that is around 130 kb. This interval is included in the one previously proposed, between 30,221,970 and 30,708,662 bp, by Sáez et al. (2017).

Progenies from plants heterozygous in the candidate region in chromosome 12 (derived from 29BL, 32BL, 22AM-GO, and 05AL) showed variable percentages of susceptible plants, from near 100% in progeny from 29BL to 58% in progeny from 22AM-GO. These progenies were not carriers of the regions associated to resistance in chromosomes 2 and 11 (Table 2), so the segregation in resistance must come from the candidate region of chromosome 12. However, the fact that the different families varied in the segregation for resistance suggested that other regions of the genome, or the different traditional genetic backgrounds, would have an effect on resistance conferred by the *locus* on chromosome 12.

Conclusions

Analysis of selected progenies recombinant in the region of chromosome 11 associated with resistance to ToLCNDV has allowed the narrowing of the candidate interval to

approximately 130 kb. The markers available in this region, along with the backcross progenies generated, would be useful in the breeding program for the introgression of this resistance into traditional genetic backgrounds. Future work will focus on the characterization of resistance coming from other genomic regions.

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Table 1. Segregation of resistant/susceptible plants in BC_1 and F_2 progenies (derived from the cross of WM-7 with different landraces derived homozygous lines) 30 days after mechanical inoculation with ToLCNDV.

		Symptoms segregation		Expected proportion	χ^2 test
Generation	Background	Resistant	Susceptible	R:S	
BC_1	11PS	22	17	1:1	0.64 (p=0.42)
	03PS	26	11	1:1	6.08 (p=0.01)
	29BL	23	13	1:1	2.78 (p=0.10)
	32BL	15	22	1:1	1.32 (p=0.25)
	22AM-GO	19	17	1:1	0.11 (p=0.74)
	05AL	26	13	1:1	4.33 (p=0.04)
F_2	02RC	27	3	3:1	3.60 (p=0.06)

* χ^2 value calculated for a dominant monogenic expected ratio (probability for the χ^2 value with one degree of freedom). p=probability of finding a value higher or equal to the χ^2.

Table 2. Genotype for the BC_1 and F_2 plants selected to evaluate their descendants. B: homozygous for 'WM-7' allele; A: homozygous for the allele of the susceptible parent; H: heterozygous.

		Generation of the progenies and genetic background						
			F_3	F_3	BC_1S_1	BC_1S_1	BC_1S_1	BC_1S_1
Markers	Position (bp)[1]	Chr[2]	02RC	02RC	29BL	32BL	22AM-GO	05AL
SNPCmND1	23,984,244	**2**	B	B	A	A	A	A
SNPCmND2	25,292,039	**2**	B	B	A	A	A	A
SNPCmND3	25,448,713	**2**	B	B	A	A	A	A
SNPCmND4	25,611,353	**2**	B	B	A	A	A	A
SNPCmND5bis	25,904,726	**2**	B	B	A	A	A	A
SNPCmND6	26,504,936	**2**	B	B	A	A	A	A
SNPCmND7	30,249,798	**11**	B	H	A	A	A	A
SNPCmND9	30,276,355	**11**	B	H	A	A	A	A
SNPCmND11	30,280,637	**11**	B	H	A	A	A	A
SNPCmND13bis	30,347,864	**11**	B	H	A	A	A	A
SNPCmND15	30,377,414	**11**	B	A	A	A	A	A
SNPCmND14	30,395,841	**11**	B	A	A	A	A	A
SNPCmND16bis	30,403,863	**11**	B	A	A	A	A	A
SNPCmND17	30,410,537	**11**	H	A	A	A	A	A
SNPCmND19	30,441,822	**11**	H	A	A	A	A	H
SNPCmND20	30,458,338	**11**	H	A	A	A	A	H
SNPCmND22	30,472,366	**11**	H	A	A	A	A	H
SNPCmND23	30,482,002	**11**	H	A	A	A	A	A
SNPCmND25	30,537,323	**11**	H	A	A	A	A	H
SNPCmND26bis	10,175,361	**12**	A	A	H	H	H	H
SNPCmND27	11,965,753	**12**	A	A	H	H	H	A
SNPCmND29	14,425,696	**12**	A	A	H	H	H	A
SNPCmND30	15,368,097	**12**	A	A	A	A	A	H
Susceptible (%)[3]			0	71	94	53	58	73

[1] Physical position in version v4 of the melon genome (available at https://www.melonomics.net/)
[2] Chromosome
[3] Percentage of susceptible plants in the descendants mechanically inoculated with ToLCNDV.

Figure 1. Symptoms after mechanical inoculation with ToLCNDV. From left to right: asymptomatic, slight, moderate and severe symptoms.