Genetic Control of the Resistance to *Zucchini yellow mosaic virus* Derived from Melon Accession IC 274006

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Introduction

Viral infections are one of the principal threats for the growth of melon (*Cucumis melo* L.) because most cultivated varieties are susceptible to several viruses. Zucchini yellow mosaic virus (ZYMV) is a potyvirus transmitted in a non-persistent manner by aphids, which causes leaf distortion, mosaic, yellowing and as a consequence reduces yield production and fruit quality (Lisa *et al.,* 1981). ZYMV was first described in Italy and nowadays it has a worldwide distribution, being one of the viruses causing some of the most important economic losses in melon (Martín-Hernández and Picó, 2021).

As insecticide application alone is not efficient for control of viruses transmitted in a non-persistent manner (Fereres, 2000), the introgression of resistance genes into commercial breeding lines is the most effective, economic, stable and environmentally respectful way to control these pathogens (Gadhave *et al.,* 2020). Availability of resistance sources is necessary to achive this objective.

The Indian accession PI 414723 has been reported as resistant to ZYMV (Pitrat and Lecoq, 1984), watermelon mosaic virus (WMV) (Gilbert et al., 1994), papaya ringspot virus (PRSV) (Pitrat and Lecoq, 1984), cucurbit aphid-borne yellows virus (CABYV) (Dogimont et al., 1996) and tomato leaf curl New Delhi virus (ToLCNDV) (López et al., 2015). The resistance to ZYMV conferred by PI 414723 has been described as either monogenic (gene *Zym*) and dominant (Pitrat and Lecoq, 1984) or oligogenic, with three complementary dominant genes: *Zym-1, Zym-2* and *Zym-3* (Danin-Poleg *et al.*, 1997). The dominant gene *Zym* has been mapped to chromosome 2 (Périn *et al*., 2002). In any case, completely resistant cultivars against ZYMV are not commercially available. Broadening the genetic base of the resistance will increase its durability in the event of appearance of new isolates that overcome the resistances available. In fact, resistance in PI 414723 has been reported as isolate-dependent (Lecoq *et al.,* 2002). Other resistance sources to ZYMV, such as IC 274014 and IC 274007 (Dhillon *et al*., 2007) or IC 274006 (Sanchís, 2018), have been described within germplasm from India. Other studies previously reported accession IC 274006 as susceptible to ZYMV and segregating for resistance to PRSV (Dhillon *et al.,* 2007). The mechanisms underlying these resistances have not been studied.

The development of resistant cultivars is especially important for organic agriculture, where the incidence of ZYMV seems to be higher (Pérez-de-Castro *et al*., 2019) and for the recovery of traditional landraces that have been displaced by elite cultivars. This is the case of the Valencian landrace 'Meló d´Or' (BGV016451), which has high organoleptic quality, but is susceptible to a wide range of pathogens, among them ZYMV.

In this work, the genetics of the resistance to ZYMV derived from melon accession IC 274006 was studied for the first time in a cross of this resistant line with the susceptible landrace BGV016451. We have also begun the genotyping of this population to identify the putative genomic regions associated with the resistance derived from this source.

Materials and Methods

In previous studies developed by the research group, the Indian melon accession IC 274006 was identified as resistant after mechanical inoculation with ZYMV (isolate ZYMV courgette, provided by GEVES-SNES). One resistant plant was selected and crossed with the Spanish susceptible melon cultivar 'Meló d´Or' (BGV016451). The F_1 generation was used to construct the whole family: F_2, $BC_{1\text{-IC}}$ and $BC_{1\text{-BGV016451}}$ (backcrosses to IC 274006 and BGV016451 respectively). Both parents (10 plants each), their F_1 (19 plants), and the segregant populations F_2 (99 plants), $BC_{1\text{-IC}}$ (78 plants) and $BC_{1\text{-BGV016451}}$ (90 plants) populations were mechanically inoculated with ZYMV. Symptoms were visually scored

according to a scale from 0 (no symptoms) to 4 (yellowing and severe mosaic symptoms) at 15-, 22- and 30-days post-inoculation (dpi). The virus infection in each plant was tested through tissue printing followed by molecular hybridization using an RNA probe specific for ZYMV, corresponding to the sequence of the viral capsid gene.

A total of 26 susceptible and 24 resistant F_2 plants were genotyped with an existing set of 124 SNPs markers evenly distributed throughout the genome and implemented for their use in the Agena Bioscience platform (Epigenetic and Genotyping unit of the University of Valencia, Unitat Central d´Investigació en Medicina (UCIM), Spain). Additionally, three IC 274006 plants as well as one BGV016451 and two F_1 plants were included in this genotyping.

Results and Discussion

All the plants of the cultivar BGV016451 were susceptible when inoculated with ZYMV (Figure 1), and it was possible to detect viral accumulation since 15 dpi. Furthermore, a high mortality rate was detected in this variety due to the viral infection. A variable response to ZYMV was observed within the accession IC 274006, as 50 % of the plants showed symptoms from 15 dpi; virus was detected in symptomatic plants of this accession. This variability observed in IC-274006 could explain that the accession was reported as susceptible to ZYMV in a previous study (Dhillon *et al*., 2007). In fact, segregation was observed in this same study for resistance to another potyvirus, PRSV (Dhillon *et al*., 2007). In any case, the offspring populations used in the work presented here were obtained from a resistant IC 274006 plant.

In the F_1 generation, 84.21 % of the plants were susceptible, which suggested recessive genetic control of the resistance. This type of control of the resistance is the most common for viruses (Truniger and Aranda, 2009) and it is usually associated with the inhibition of virus multiplication and/or movement. For example, several recessive mutations in the translation initiation factors eIF4E and eIF(iso)4E confer resistance to potyvirus infection in several hosts (Robaglia and Caranta, 2006). The fact that 15.79% of the plants in the F_1 generation were resistant suggested an incomplete penetrance of the resistance gene (Table 1).

In the F_2 population, 72 plants showed moderate to very severe symptoms (scores 2-4) with high viral accumulation, so they were considered susceptible (Figure 1, Table 1). The rest of the plants showed light or an absence of symptoms (scores 0-1) and viral accumulation was low or undetectable; therefore, these plants were classified as resistant. $BC_{1\text{-IC}}$ also showed segregation of symptoms as 37 plants were found to be resistant (scores 0-1) and 41 susceptible (scores 2-4). The $BC_{1\text{-BGV016451}}$ population also segregated for symptom severity with 12 resistant (scores 0-1) and 78 susceptible (scores 2-4) plants, supporting incomplete penetrance. In both BC generations, viral accumulation also supported the visual evaluation of each plant.

Considering the incomplete penetrance observed in the F_1 generation, measured as the percentage of resistant plants in heterozygotes for the resistant gene (p=0.15789), the segregation observed in F_2 and both BC generations fit the expected ratio for a single recessive gene (Table 1).

To determine the contribution of different genomic regions to ZYMV resistance, 24 resistant and 22 susceptible F_2 plants were genotyped, along with both parents and their F_1 offspring. The results indicated that a genomic region in chromosome 5 could be related to the resistance, because a significant difference was found in the phenotypic response between the different genotypes for one of the SNPs located in this region (*p-value* = 0.00194). The highest concentration of resistance genes in the melon genome is located in chromosome 5 (González *et al.*, 2013), in a region including that proposed here as associated to IC 274006-derived resistance to ZYMV. Further work will be carried out, including quantification of virus titer in resistant and susceptible plants as well as a wider genotyping, to confirm these results.

Conclusion

The resistance to ZYMV derived from IC 274006 could be used in combination with resistance derived from other sources, to achieve a more durable resistance against ZYMV and increase its level. The molecular markers identified here and the generations available will be useful to continue the breeding program for the introgression of the resistance derived from this source in the 'Meló d'Or' genetic background and in other commercial types.

Acknowledgements

This research was funded by the Spanish Ministerio de Ciencia, Innovación y Universidades, grants number AGL2017-85563-C2 (1-R) and RTA2017-00061-C03-03 (INIA), and by the Conselleria d'Educació, Investigació, Cultura i Esports de la Generalitat Valenciana, grant number PROMETEO/2017/078 (to promote excellence groups, cofinanced with FEDER funds). M.L. is a recipient of a predoctoral fellowship (PRE2018-083466) of the Spanish Ministerio de Ciencia, Innovación y Universidades. The authors would like to thank M. García for her help in the data evaluation. Authors also thank the UPV Genebank for providing seeds of Spanish traditional melons.

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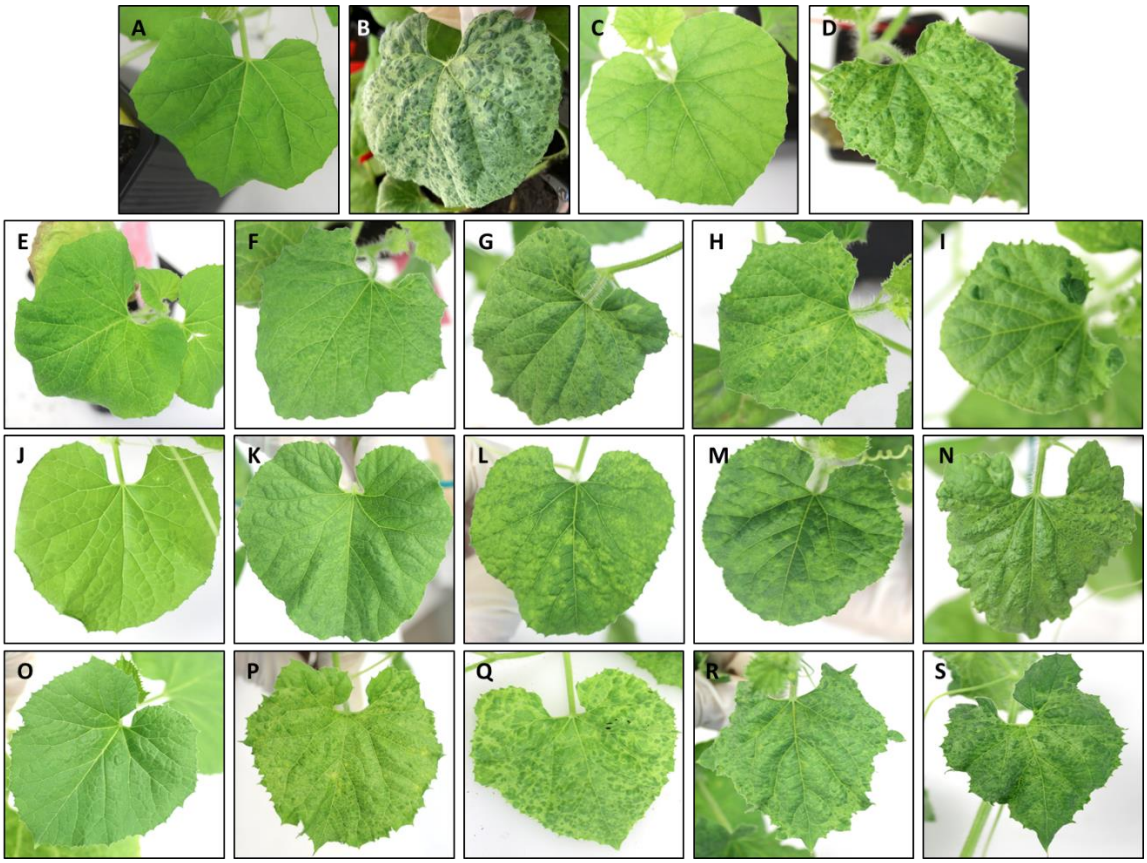

Figure 1. Absence of symptoms in the resistant accession IC 274006 (A) and severe mosaic symptoms caused by *Zucchini mosaic virus* (ZYMV) in the susceptible cultivar 'Meló d´Or' BGV016451 (B). Diversity of symptoms caused in the F_1 (C-D), F_2 (E-I), $BC_{1\text{-IC}}$ (J-N) and $BC_{1\text{- BGV016451}}$ (O-S) generations.

Table 1. Segregation of resistant/susceptible plants in F_1, F_2, $BC_{1\text{-IC}}$ and $BC_{1\text{-BGV016451}}$ offspring derived from the cross IC 274006 x BGV016451. R: resistant; S: susceptible.

Progeny	Phenotype	Frequency	Observed segregation	Expected segregation	X^2 test *
F_1	R	p	3	3	--
	S	1-p	16	16	
F_2	R	0.25 + 0.5p	27	32.57	1.417 (0.233)
	S	0.25 + 0.5(1-p)	72	66.43	
$BC_{1\text{-IC}}$	R	0.5 + 0.5p	37	45.16	3.5 (0.061)
	S	0.5(1-p)	41	32.84	
$BC_{1\text{- BGV016451}}$	R	0.5p	12	7.11	3.661 (0.056)
	S	0.5 + 0.5(1-p)	78	82.89	

* chi-square value calculated for a recessive monogenic expected ratio (probability for the chi-square value with one degree of freedom)