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Additional Information

Evaluation of *Cucurbita moschata* x *Cucurbita maxima* Rootstocks against ToLCNDV

M. Ferriol¹, C. Sáez², C. Gisbert², C. López² and B. Picó²

¹Instituto Agroforestal Mediterráneo. Universitat Politècnica de València, Valencia, Spain. ²Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV-UPV), Universitat Politècnica de València, Valencia, Spain.

Abstract

Tomato leaf curl New Delhi virus (ToLCNDV) was first detected in Spain in 2012-2013. Currently, it causes major economic losses in zucchini (*Cucurbita pepo*), and until now no resistant or tolerant sources have been found in this species. Although in general less harmful, this begomovirus also infects other *Cucurbita* species, such as *C. maxima* and *C. moschata*, whose hybrids are commonly used as rootstocks for grafting watermelons and melons. In the present study, we have screened a selection of *C. maxima* and *C. moschata* accessions and their corresponding hybrids against ToLCNDV using mechanical inoculation. Symptoms were scored at 15 and 30 days post inoculation (DPI), and the Vulnerability Index (Vul) was calculated. ToLCNDV was detected by PCR. At 30 DPI, all the *C. moschata* accessions had lower Vuls than the *C. maxima* accessions, which were more susceptible. This is consistent with previous studies that showed higher levels of resistance in some accessions of *C. moschata*. All accessions were ToLCNDV positive by PCR. Most of the evaluated hybrid progenies showed severe infections similar, or even more severe, to those found in their parentals. The great aggressiveness of this virus and the high susceptibility of the assayed hybrids can make the production of rootstocks difficult. Furthermore, the use of susceptible rootstocks to ToLCNDV can contribute to increase the severity of the infection in scions. Therefore the development of new rootstocks derived from selected tolerant *C. moschata* and *C. maxima* accessions might minimize this effect.

Keywords: *Cucurbita* hybrid, Mechanical inoculation, Squash, Vulnerability Index

INTRODUCTION

Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus (Geminiviridae) first reported in India and neighboring countries. This virus causes great economic losses in zucchini (*Cucurbita pepo*) in southeastern Spain since 2012 (Juárez et al., 2014).

Mechanical transmission of one Spanish isolate was successfully used to screen for resistance in different Cucurbitaceae genera, including accessions of the most important crops within *Cucurbita*, *Cucumis* and *Citrullus* (squashes, melons, cucumbers, and watermelons). Some melon (*Cucumis melo*) accessions were found with acceptable levels of tolerance. However, all the assayed accessions of *C. pepo* were highly susceptible. Other *Cucurbita* species, such as *C. maxima* and *C. moschata*, were less susceptible. The former showed, in general, delayed symptoms evolving to moderate or severe, and the latter included accessions with variable response, from tolerant to highly susceptible (López et al., 2015).

Both *C. maxima* and *C. moschata* have less economic importance as vegetables than *C. pepo*, but they have been selected as preferred parents to generate commercial hybrid rootstocks for other cucurbits, mainly used for watermelon and increasingly for melon and cucumber (King et al., 2010).

The use of rootstocks susceptible to ToLCNDV could potentially affect scions, as rootstocks susceptible to *Cucumber Green Mottle Virus* CGMV do (Lee et al., 2010). In the present work, we assess the response of several *C. maxima* and *C. moschata* parentals and their corresponding hybrids to ToLCNDV, using mechanical inoculation, and discuss strategies to improve the tolerance of new rootstocks to this virus.

MATERIALS AND METHODS

Eight F1 *C. maxima* x *C. moschata* hybrid progenies were used in the present study. These were generated in a previous assay in which a large number of genotypes of both species, representing taxonomic and geographic diversity, were crossed to select inter-specific combinations with synchronized flowering and cross compatibility, useful for rootstock production (Table 1).

Table 1. Name and origin of the *C. maxima* and *C. moschata* accessions used as parentals for the F1 hybrids screened for ToLCNDV tolerance.

<i>Cucurbita maxima</i>		<i>Cucurbita moschata</i>	
Accession	Origin	Accession	Origin
AN-CU-59	Spain	PI550689	USA
VAV4381	Peru	AFR-CU-1	Morocco
SUD-CU-6	Argentina	PI264551	Guatemala
VAV1860	Australia	PI419083	China
VAV2422	South African Republic	PI369346	Costa Rica
VAV3202	Chile		

PI genotypes were kindly provided by U.S. Dept. Agric., National Plant Germplasm System (USDA-NPGS), while VAV genotypes were provided by Vavilov Institute of Plant Genetic Resources (VIR).

For comparative purposes, three commercial rootstocks (one *C. moschata* x *C. maxima* F1 hybrid, one *C. maxima* x *C. maxima*, and one *Lagenaria siceraria*) were also included in the analysis.

Five to 10 plants per accession and F1 hybrid progeny were mechanically inoculated with a Spanish isolate of ToLCNDV, according to the procedure described in López et al. (2015). Symptoms were scored at 15 and 30 days post inoculation (DPI) using a scale from 0 (absence of symptoms) to 4 (very severe symptoms or dead plant). ToLCNDV presence was confirmed by PCR. A Vulnerability Index (Vul) was calculated according to López et al. (2015) for each accession and symptoms score. On the basis of this index, and similarly to the classification used by Islam et al. (2011), we considered as tolerant genotypes whose Vul = 0 – 25 %, moderately susceptible Vul= 26 – 50 %, susceptible Vul= 51 – 75 %, and highly susceptible Vul= 76 – 100 %.

RESULTS AND DISCUSSION

Parentals

Although more variable at 15 DPI, at the end of the assay (30 DPI), the *C. moschata* accessions had lower Vuls than the *C. maxima* accessions, which were more susceptible (ranging from 45 to 93% and from 75 to 100%, respectively) (Figs. 1 and 2). This is consistent with previous studies that showed higher levels of tolerance in some accessions of *C. moschata* than in *C. maxima* (Sohrab et al., 2010; López et al., 2015). All accessions were ToLCNDV positive by PCR. According to Vul, all the *C. maxima* parentals were classified as highly susceptible, except VAV1860 that was susceptible, whereas only one *C. moschata* was highly susceptible (PI419083), three susceptible (AFR-CU-1, PI264551, and PI369346) and one moderately susceptible (PI550689).

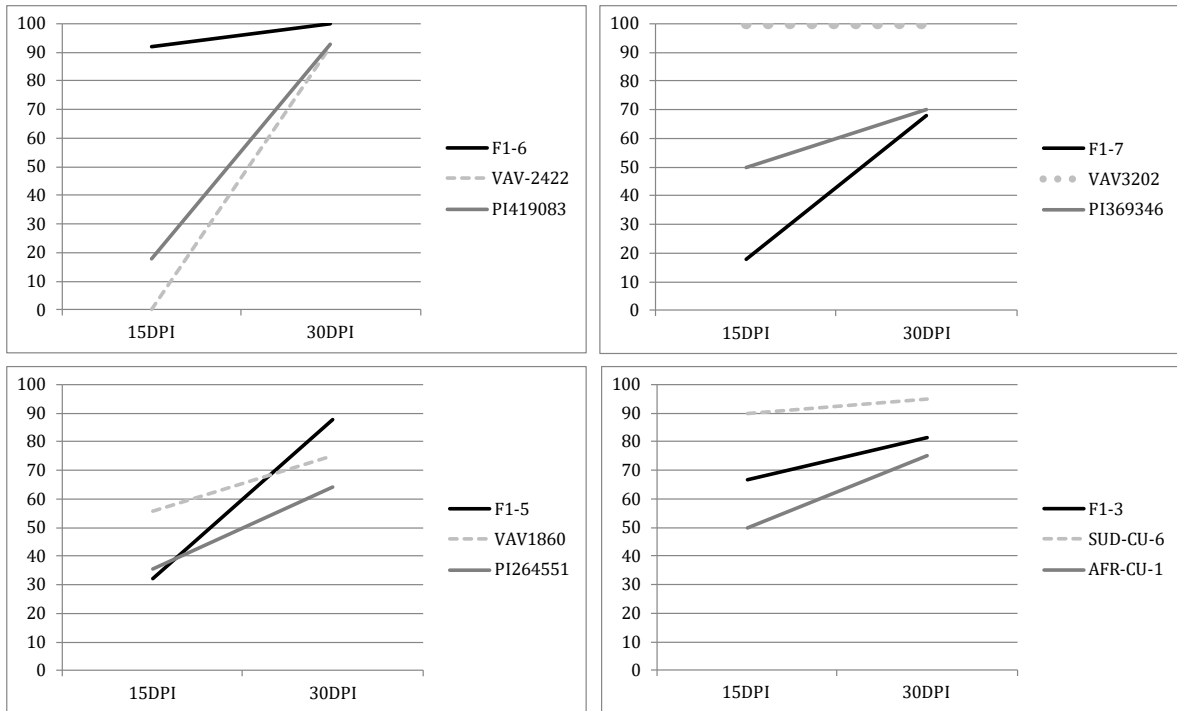


Figure 1. Evolution of the Vulnerability Index at 15 and 30 days ToLCNDV post - inoculation (DPI) of 4 F1 hybrids derived from the cross *Cucurbita maxima* x *C. moschata* accessions (highly susceptible or susceptible to ToLCNDV) and their parents. Hybrids are depicted in black, *C. maxima* in light grey and *C. moschata* in dark grey. Dotted lines represent female parents.

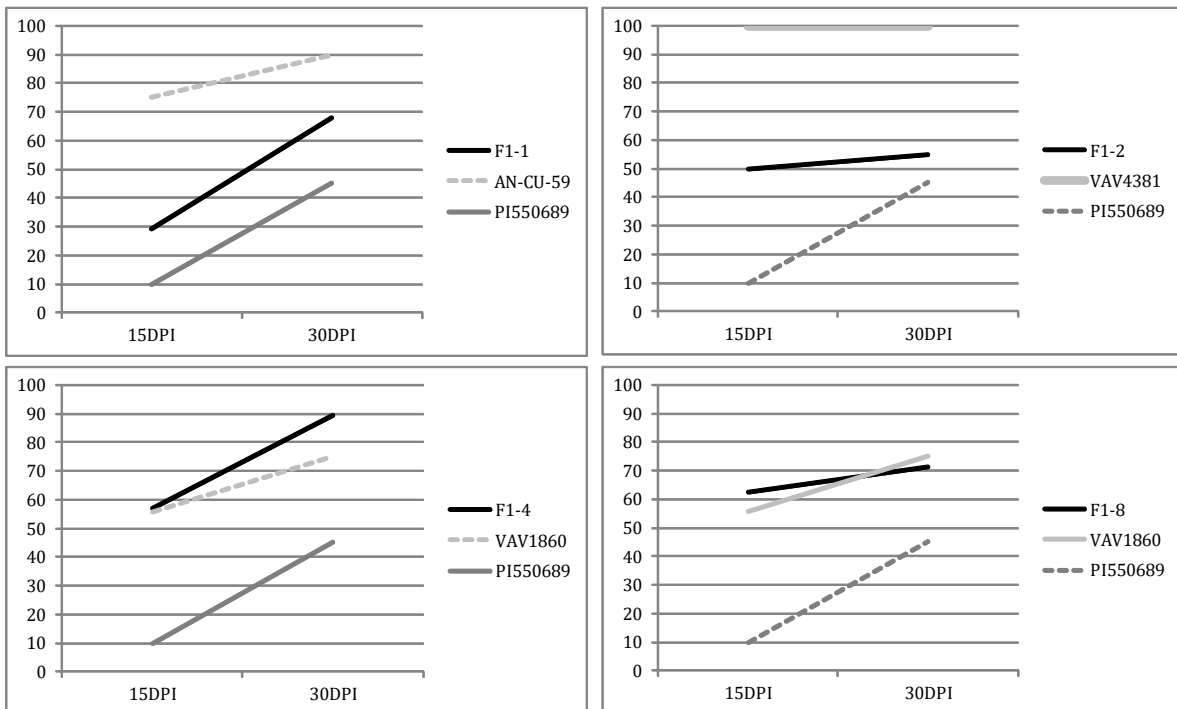


Figure 2. Evolution of the Vulnerability Index at 15 and 30 days ToLCNDV post - inoculation (DPI) of 4 F1 hybrids derived from the cross *Cucurbita maxima* x *C. moschata* PI 550689 (moderately susceptible to ToLCNDV) and their parents. Hybrids are depicted in black, *C. maxima* in light grey and *C. moschata* in dark grey. Dotted lines represent female parents.

Hybrid Progeny

Vulnerability Indexes of the F1 hybrid progenies ranged from 55 to 100%, showing in all cases moderate to very severe ToLCNDV symptoms (Figures 1 and 2) and positive PCR amplifications (data not shown). The two commercial F1 hybrids, which are currently used as rootstocks for cucurbits, showed a Vul of 100% at 30 DPI, similar to some of the progenies used here. The *Lagenaria siceraria* commercial rootstock had a Vul of 85.7%, showing severe symptoms according to previous studies that reported a high susceptibility in this species (Sohrab et al., 2010; López et al., 2015).

The hybrid behavior was more similar to the response of the *C. moschata* than to the *C. maxima* parental, although ToLCNDV infection in hybrids was, in general, more severe than in the corresponding *C. moschata* at 30 DPI. The hybrid derived from the highly susceptible *C. moschata* parental (PI 419083) behaved as highly susceptible (Vul at 30 DPI=100%), whereas those derived from the susceptible *C. moschata* accessions (AFR-CU-1, PI264551, and PI369346) ranged from susceptible to highly susceptible (Vul at 30 DPI=70-90%), even in crosses with highly susceptible *C. maxima* parentals (such as VAV3202).

We assayed several crosses derived from the *C. moschata* PI550689 that was highly compatible with different *C. maxima* accessions and was only moderately susceptible to ToLCNDV (Vul<50%) (Figure 2). The derived hybrids were less susceptible (Vul at 30 DPI=50-70%) than those of the previous crosses. The direction of the cross seems to influence the response of the hybrid. When *C. moschata* was used as female parent, hybrid Vuls were lower than when it was used as male parent. This was more obvious when the same parental accessions were used, thus *C. maxima* VAV1860 x *C. moschata* PI550689 originated a progeny with a Vul of 89.3% while the reciprocal was 71.4% (Figure 2).

CONCLUSIONS

The great aggressiveness of ToLCNDV in cucurbits, mainly in the *Cucurbita* genus, can make the production of rootstocks difficult as the use of susceptible rootstocks can contribute to increase the severity of the infection in scions (Guan et al., 2012). We have found that the response of the hybrid to the viral infection can be improved using selected *C. moschata* accessions as female parental in the crosses. In the current assay, accessions were selected for cross compatibility and later assayed for resistance. New screenings have provided *C. moschata* accessions with higher levels of tolerance to ToLCNDV that can be assayed for their cross compatibility in interspecific crosses and for the production of new hybrids with improved levels of tolerance to this virus. Also, the identification and use of more tolerant *C. maxima* accessions can minimize the effect of the virus.

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