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Additional Information

Tolerance to ToLCNDV in *Cucurbita* spp.

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Abstract

Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus (Geminiviridae) firstly reported in India and neighboring countries. A severe outbreak of ToLCNDV in Zucchini (*Cucurbita pepo*) occurred in Southern Spain in 2012. Mechanical inoculation and natural infection with *Bemisia tabaci*, in Almeria, were used to screen part of the COMAV's *Cucurbita* core collection. The tolerance was assessed by symptomatology and by a PCR-based diagnostic method that allows the detection of the two viral components in a single reaction. Mechanical transmission of ToLCNDV was confirmed in all the species. Severe symptoms were found 30 and 35 days after mechanical and natural inoculation, respectively, in *C. pepo*, in all accessions belonging to the Zucchini morphotype and to other morphotypes of the subsp. *pepo*, subsp. *ovifera* and even in the wild relative *C. fraterna*. *Cucurbita maxima* and *C. ficifolia* were also highly susceptible. The only species showing tolerance was *C. moschata*. Instead of curling, which was typically observed in most *Cucurbita* species, in *C. moschata* we observed mosaic and mottling, which suggest a differential response of this species. Under natural infection, a few *C. moschata* accessions remained asymptomatic and no virus was detected in the plant apex. Under mechanical inoculation, ToLCNDV symptoms were mild and delayed in these accessions and they were PCR positive. Some of the more tolerant accessions came from India.

Keywords: Resistance, *Tomato leaf curl New Delhi virus*, Mechanical inoculation, Zucchini, Whitefly transmission.

INTRODUCTION

Cucurbits are affected by different pathogens, which cause high yield losses. A new viral disease appeared in 2012 in Murcia and Almería (Spain) affecting zucchini crops (*C. pepo* subsp. *pepo*) and other important cucurbits, such as melon (*Cucumis melo*), cucumber (*Cucumis sativus*) and watermelon (*Citrullus lanatus*). The causal agent was the begomovirus *Tomato leaf curl New Delhi virus* (ToLCNDV), firstly reported in 1995 in tomato plants from India (Srivastava et al., 1995).

ToLCNDV is one of the most serious threats to cucurbit production in Spain. The use of resistant varieties is an economic and environmentally friendly method to control viral diseases. Islam et al. (2011) reported resistance in some accessions of *Luffa cylindrica*, a severely affected crop in India. More recently, López et al. (2015) developed a method for the mechanical inoculation of the Spanish isolate of ToLCNDV. Using this protocol, ToLCNDV could be transmitted to a selected germplasm collection of the genera *Cucumis*, *Citrullus*, and *Cucurbita*.

In that screening, some wild and exotic melon genotypes, all from India, were tolerant to the infection. Regarding the *Cucurbita* species, a differential response was found. The accessions of *Cucurbita pepo* evaluated in that preliminary assay were highly susceptible, whereas the infection was less severe in *Cucurbita maxima* and *Cucurbita moschata*. Those

results are promising and suggest that the huge variability within the *Cucurbita* genus (Ferriol et al., 2003) could be exploited to identify natural variants with resistance to ToLCNDV.

Similarly to other begomoviruses, ToLCNDV has the whitefly *Bemisia tabaci* as natural vector of transmission (Chang et al., 2010). Therefore, the genotypes found to be resistant/tolerant to this virus (López et al., 2015) using mechanical inoculation were also assayed under natural infection conditions.

The aim of this study was the screening of a wide collection of selected accessions of *Cucurbita* species against ToLCNDV, using sap transmission and natural infection.

MATERIAL AND METHODS

In this work, 104 accessions of the *Cucurbita* genus were evaluated. This collection included genotypes of diverse origins, belonging to the three main cultivated species of the genus (*C. pepo*, *C. maxima* and *C. moschata*). The genotypes of *C. pepo* assayed represented the main morphotypes of the two subspecies (subsp. *ovifera* and subsp. *pepo*). One accession of *C. ficifolia* was also included, as well as two accessions of *C. fraterna*, which has been proposed as the wild ancestor of *C. pepo* (Table 1).

Table 1. Species, subspecies, morphotype and origin of the *Cucurbita* accessions screened for ToLCNDV resistance.

Species ^a	Subspecies ^a	Morphotypes	Number of accessions	Origin	
<i>pepo</i>	<i>pepo</i>	Pumpkin	19	Guatemala, Spain, Hungary, Italy, Turkey	
		Vegetable Marrow	24	Morocco, Spain	
		Zucchini	8	Spain	
		Cocozelle	13	Spain, Italy, Greece	
	<i>ovifera</i>	Acorn	4	USA	
		Scallop	2	USA	
		Croockneck	1	Spain	
		Ornamentals	2	Spain	
		<i>fraterna</i>		2	Mexico
		<i>maxima</i>		14	African Republic, Angola, Argentina, Chile, Colombia, Morocco, Peru, Spain
<i>moschata</i>		14	Canada, China, Cuba, Costa Rica, Ecuador, Guatemala, India, Japan, Nigeria, Dominican Republic, Spain, USA, Zimbabwe		
<i>ficifolia</i>		1	Peru		

^a *Cucurbita pepo* L. ssp. *pepo*, *Cucurbita pepo* L. ssp. *ovifera* (L.) D.S. Decker (= *texana* var *ovifera*), *Cucurbita fraterna* L.H. Bailey, *Cucurbita moschata* Duchesne, *Cucurbita maxima* Duchesne.

All the accessions were screened for ToLCNDV resistance by mechanical inoculation in a growth chamber as described by López et al. (2015). Young leaves of symptomatic infected zucchini plants from Almería were used as source of inoculum. The same accessions were assayed by natural transmission of the virus with viruliferous whiteflies in a greenhouse in Almería. Infected adult plants of the *C. pepo* susceptible cultivar “Sinatra” with clear symptoms

of ToLCNDV (confirmed by PCR), along with healthy seedlings of the same cultivar, were introduced into the greenhouse to establish a population of viruliferous whiteflies. Once healthy plants started to show symptoms, seedlings of the different *Cucurbita* accessions were distributed in the greenhouse and kept till the end of the assay. All tested plants were visually evaluated and scored for symptoms severity according to a scale previously established by López et al. (2015) (ranging from 0: absence of symptoms to 4: very severe symptoms or dead plants). Evaluation of symptoms was done at 15 and 30 days post mechanical inoculation (dpi), and at 35 dpi after whitefly transmission, respectively. Semi-quantitative PCR was used to detect ToLCNDV and to verify the infection in those accessions with more promising behaviors at 15 and 30 days after mechanical inoculation. Some susceptible accessions were added to the semi-quantitative PCR test as controls.

RESULTS AND DISCUSSION

Despite the high diversity assayed of *C. pepo*, all the morphotypes of subsp. *pepo* (Pumpkin, Vegetable Marrow, Zucchini and Cocozelle) developed severe symptoms after both mechanical inoculation and whitefly transmission. The response of subsp. *ovifera* was similar, and both cultivated and ornamental morphotypes were highly susceptible. Mean symptom scores ranged from 3.5 to 4 within this species (Table 2). Leaf curling, mosaic, yellowing and short internodes were observed.

Table 2. Mean and range of symptoms scored at 15/30 days after mechanical inoculation and 35 days after whitefly transmission. Symptoms were scores according to the scale 0 (absence of symptoms); 1 (mild symptoms); 2 (moderate symptoms); 3 (severe Symptoms); 4 (very severe symptoms or dead plant).

Species ^a	Subspecies ^a	Morphotypes	Mechanical inoculation				Whitefly transmission	
			15dpi		30dpi		35dpi	
			Mean	Range	Mean	Range	Mean	Range
<i>pepo</i>	<i>pepo</i>	Pumpkin	2.1	(0-4)	3.5	(1-4)	4.0	(4)
		Vegetable Marrow	1.5	(0-4)	3.7	(1-4)	3.9	(4)
		Zucchini	2.8	(0-4)	3.5	(2-4)	4.0	(4)
		Cocozelle	2.2	(0-4)	3.5	(1-4)	4.0	(4)
	<i>ovifera</i>	Acorn	3.0	(0-4)	3.8	(2-4)	4.0	(4)
		Scallop	2.6	(0-4)	3.6	(2-4)	4.0	(4)
		Crookneck	2.8	(0-4)	4.0	(4-4)	4.0	(4)
		Ornamentals	1.9	(0-4)	3.9	(3-4)	4.0	(4)
<i>fraterna</i>		2.0	(0-4)	3.8	(3-4)	3.5	(3-4)	
<i>maxima</i>		2.1	(0-4)	3.1	(0-4)	4.0	(4)	
<i>moschata</i>		1.1	(0-4)	1.8	(0-4)	2.4	(0-4)	
<i>ficifolia</i>		4.0	(4)	4.0	(4)	4.0	(4)	

^a *Cucurbita pepo* L. ssp. *pepo*, *Cucurbita pepo* L. ssp. *ovifera* (L.) D.S. Decker (= *texana* var *ovifera*), *Cucurbita fraterna* L.H. Bailey, *Cucurbita moschata* Duchesne, *Cucurbita maxima* Duchesne.

C. fraterna is a wild species native from America, from the center of origin of *C. pepo*. The two accessions assayed in this study displayed a susceptible response similar to that of the domesticated genotypes (mean symptom scores of 4) (Table 2). The only evaluated

accession of *C. ficifolia* was very susceptible at the end of the assay by using the two inoculation methods.

Early symptoms found in *C. maxima* accessions were less severe than those of *C. pepo*. However, they evolved to very severe at the end of the assay (Table 2). Leaf decay and a more intense yellowing were characteristic symptoms in this species.

Variability of *C. moschata* accession responses to natural and mechanical ToLCNDV infection was observed. Most of the accessions assayed were susceptible, but according to previous studies (López et al., 2015), some of them remained symptomless or developed only mild symptoms after infection (Table 2). *C. moschata* has been previously described as source of resistance to viruses (Paris and Kabelka, 2009), and, in this case, the accession with the best behavior was collected in India, where virus and plants could have co-evolved. ToLCNDV was successfully detected by semi-quantitative PCR in all accessions evaluated. Viral accumulation increased from 15 to 30 dpi in all cases, except in *C. moschata*. In the more resistant/tolerant accessions of this species the amount of virus seems to decrease as the infection progresses (Figure 1). Despite the plants remained symptomless we found an intermediated level of viral accumulation.

Virus acumulation in Cucurbita accessions

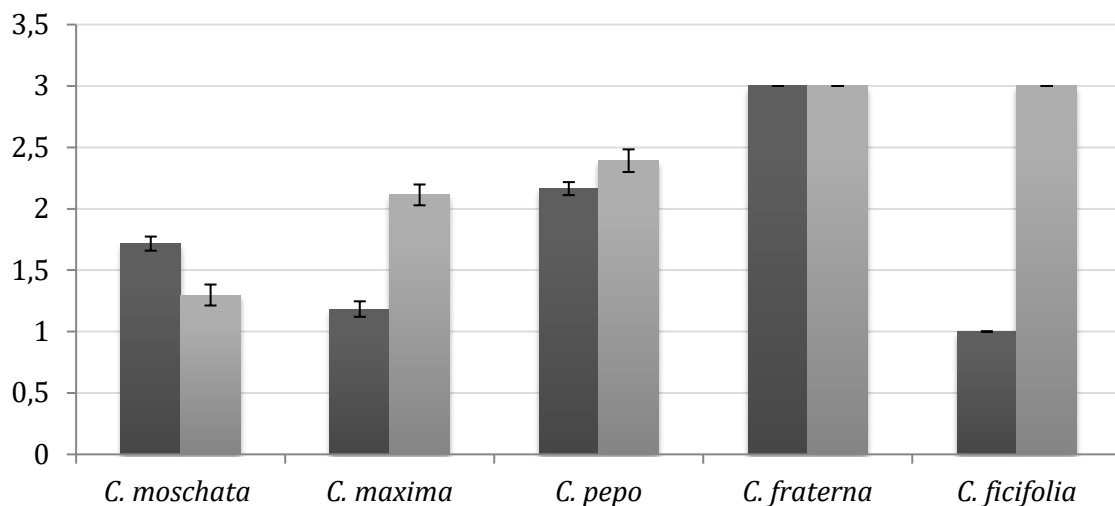


Figure 1. Mean and standard error of viral accumulation (scores from semi-quantitative PCR results as 1 low, 2 intermediate, 3 high) in several genotypes of *C. maxima*, *C. moschata*, *C. pepo*, *C. ficifolia* and *C. fraterna* with different levels of resistance to ToLCNDV at 15 (dark grey bars) days post inoculation (dpi) and 30 (dpi) (light grey bars).

The use of these tolerant *C. moschata* accessions, symptomless even after natural transmission of the virus, could be a promising approach to obtain cultivars of *C. pepo* with tolerance to ToLCNDV.

CONCLUSIONS

ToLCNDV has become a serious threat to cucurbits cultivation. *C. pepo* is the main affected crop and the collection screened in this work was fully susceptible. Even the wild ancestor *C. fraterna* was very susceptible to the infection. *C. ficifolia* and *C. maxima* had also highly severe infections, mainly after whitefly transmission. We only found resistance/tolerance among the *C. moschata* accessions. Two accessions were symptomless and had low viral concentrations. These accessions will be used in the generation of segregant populations to study the genetic basis of such a resistance and to introgress it in commercial cultivars of *C. pepo*.

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