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Research Paper

Assessment of resistance of *Ty-1* and *ty-5* genes in *Solanum lycopersicum* plants infected with tomato yellow leaf curl virus (TYLCV)

José Ángel Cabrera [a], Pedro Carbonell [a], Aranzazu Alonso [a], Clara Pérez-Moro [b], Ana Pérez de Castro [b], Juan José Ruiz [a], Santiago García-Martínez [a,*]

[a] *Instituto de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Universidad Miguel Hernández, ctra. de Beniel km 3.2, 03312, Orihuela, Alicante, Spain*

[b] *Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Camino de Vera s/n, 46022, Valencia, Spain*

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ABSTRACT

A group of viruses within the *Begomovirus* genus is responsible for tomato yellow leaf curl virus disease (TYLCVD). The genus *Begomovirus* belongs to the family *Geminiviridae*, which is divided into 14 genera. TYLCV is a virus that since its emergence in 1939 continues to severely affect tomato crops worldwide, partly due to its ease of recombination and emergence of new mutations. The virus is transmitted by the whitefly (*Bemisia tabaci*), and since measures to control *B. tabaci* populations are not fully effective and there is always a risk of infection, the best defence mechanism is genetic resistance. Two sets of breeding lines with different homozygous combinations of the *Ty-1* and *ty-5* genes (*Ty-1* lines, *ty-5* lines, *Ty-1/ty-5* lines and susceptible lines) were studied. A priori, this is the first study of TYLCV inoculation under controlled conditions in lines carrying the *Ty-1* and *ty-5* genes. The aim of the study was to evaluate the behaviour of tomato breeding lines carrying these two genes and test their level of tolerance to TYLCV. All plants showed a homogeneous response depending on the genotype studied. The results of the breeding lines studied with all homozygous combinations of the *Ty-1* and *ty-5* genes were consistent with those of the controls. Susceptible genotypes (ss/SS) showed severe symptoms and high viral accumulation, and resistant genotypes (*Ty-1/ty-5, ty-5* and *Ty-1*) showed very mild symptoms and low viral accumulation. The phenotypic responses to TYLCV can be classified into two groups: the susceptible genotypes (ss/SS) for the two genes and the resistant genotypes (*Ty-1, ty-5* and *Ty-1/ty-5*), presenting *Ty-1, ty-5* or both. In this study, we have demonstrated that both *Ty-1* and *ty-5*, whether used together or individually, provide a similarly high level of resistance.

1. Introduction

Most viruses infecting plants (80–90%) have single-stranded RNA as their genetic component, while the remaining plant pathogenic viruses contain DNA as their genome (Rojas, 2000). The *Geminiviridae* family of viruses has a DNA genome, composed of one or two small circular, single-stranded molecules (DNA-A and DNA-B), approximately 2.5 to 3.0 kb each. With a total genome size ranging from 2.5–5.0 kb, geminiviruses are among the smallest viruses with independently replicating genomes and are among the only DNA viruses with a split genome (Rojas, 2000). Based on host range, type of insect vector, genome organization, and phylogenetic relationships, the *Geminiviridae* family is divided into fourteen genera: *Becurtovirus, Begomovirus, Capulavirus, Citlodavirus, Curtovirus, Eragrovirus, Grablovirus, Maldovirus, Mastrevirus, Mulcrilevirus, Opunvirus, Topocuvirus, Turncurtovirus* and *Topilevirus*. There are 520 species in the *Geminiviridae* family. Among these species, 445 belong to the genus *Begomovirus*, which is the largest in the entire virosphere (Silva et al., 2023). A group of viruses within the *Begomovirus* genus is responsible for tomato yellow leaf curl virus disease (TYLCVD) (Picó et al., 1996). Begomoviruses possess one or two circular single-stranded DNA (ssDNA) genome(s), each about 2.7–2.8 kb in size. TYLCV and most TYLCV-like begomoviruses have monopartite genomes consisting of one ssDNA molecule, except for tomato yellow leaf curl Kanchanaburi virus (TYLCKaV) and tomato yellow leaf curl Thailand virus (TYLCTHV). These two begomoviruses are bipartite, with a genome containing two ssDNA molecules—DNA-A and DNA-B (Yan et al., 2021). TYLCV has great potential to change due to factors such as mutation and genetic recombination, enabling rapid adaptation of the

* Corresponding author.
E-mail address: sgarcia@umh.es (S. García-Martínez).

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TYLCV complex to ever-changing environmental conditions (Navas-Castillo et al., 2011; Yan et al., 2021). A high degree of intra- and inter-species recombination has been observed within the TYLCV complex and among begomoviruses (Abhary et al., 2007; Yan et al., 2021). For example, TYLCV-IL is the result of recombination between TYLCV-Mld and tomato leaf curl Karnataka virus (ToLCKV), while the Sardinia strain (TYLCSV) likely emerged from a South African cassava mosaic virus (SACMV) ancestor by genetic exchange through recombination. Co-infection of tomato plants with TYLCV and TYLCSV led to the emergence of two recombinant viruses associated with TYLCD — TYLCMaV and TYLCAxV —which have acquired a broader host range than any of the parents. Moreover, a new virus strain, TYLCV-IS76, has emerged due to a recombination event between TYLCV-IL and the Spanish strain of TYLCSV (TYLCSV-ES). TYLCV-IL [IT:Sic23:16] and TYLCV-IL-[IT:Sar IS141:16] originated from the genetic exchange of parental strains TYLCV and TYLCSV (Yan et al-. 2021).

The disease is transmitted via the tobacco whitefly, *Bemisia tabaci* Gennadius, (Cohen and Harpaz, 1964). Symptoms include dwarfism, upward leaf puckering, vein clearing, and excessive branching and stunting, all associated with mild to severe mosaic symptoms and partial or total plant sterility (Vasudeva and Sam Raj, 1948). The virus infects dicotyledons, mainly Solanaceae, and causes serious problems in tomato crops in tropical and subtropical regions around the world, and has even been known to wipe out entire harvests (Picó et al., 1996; Rojas et al., 2005; Wang et al., 2018; Kill et al., 2021). The first evidence of economic damage to vegetable crops caused by *B. tabaci* was recorded in 1931 in Israel. A TYLCV-like disease was first reported in Israel in 1939–1940 and was associated with outbreaks of *B. tabaci* in the Jordan and Bet She'an Valleys; the entire tomato crop was completely destroyed (Cohen and Antignus, 1994). In Spain, the virus was first detected in 1992 in greenhouses in Murcia (Moriones et al., 1993) and Almería (Reina et al., 1994). It belonged to the species from Sardinia (tomato yellow leaf curl Sardinia virus, TYLCSV). Isolates of the TYLCV-Sr and TYLCV-IL species (renamed TYLCSV and TYLCV respectively; Fauquet et al., 2000) were found to be involved in TYLCV epidemics in these same regions (Sánchez-Campos et al., 1999). Five years later, following more severe episodes, the presence of the tomato yellow leaf curl virus (TYLCV) species was detected in 1997 (Navas-Castillo et al., 1997). TYLCV-IL is probably the most widespread strain worldwide (Lefeuvre et al., 2010). The high prevalence of TYLCV-IL in southern and southeastern Spain is due to the progressive displacement of TYLCV-Sr. This may be attributed to two main factors. Firstly, TYLCV-IL is more efficiently transmitted by local *B. tabaci* biotypes. Secondly, the common bean, which serves as a bridge crop between tomato crops, acts as a host for TYLCV-IL but not TYLCV-Sr. This suggests that the common bean serves as a reservoir for TYLCV-IL (Sánchez-Campos et al., 1999). Currently, TYLCV continues to hinder the cultivation of both commercial and traditional varieties. Since preventive and control measures against *Bemisia tabaci* populations are not fully effective, genetic resistance is the best long-term strategy for managing the disease. In fact, genetic resistance is emerging as the best strategy for controlling viral diseases in general due to an increasingly demanding international market and a growing emphasis on environmental sustainability (Cabrera et al., 2024).

To date, six TYLCV tolerance loci have been identified. Zamir et al. (1994) found *Ty-1* on chromosome six of *S. chilense* accession LA1969. *Ty-2*, derived from *S. habrochaites*, was later mapped to chromosome 11 (Hanson et al., 2000; Hanson et al., 2006). *Ty-3* was located on chromosome six of *S. chilense* accessions LA1932 and LA2779 (Ji et al., 2007) near the *Ty-1* locus, suggesting a genetic link between *Ty-1* and *Ty-3* (Ji et al., 2007a). In 2013, Verlaan et al. (2013) accurately mapped *Ty-1* and *Ty-3,* concluding that the *Ty-3*-assigned region of approximately 71 kb overlapped with the *Ty-1*-containing region. The *Ty-4* gene was also mapped in *S. chilense* accession LA1932, but on chromosome three (Ji et al., 2009). Later, in another study designed to map the loci controlling TYLCV resistance in TY172, researchers identified a recessive QTL called *Ty-5* near the *SlNAC1* marker on chromosome four (Friedmann et al., 1998; Anbinder et al., 2009). The University of Florida's tomato breeding programme has developed numerous breeding lines with Begomovirus resistance derived from Tyking, a hybrid bred by Royal Sluis (Enkhuizen, Netherlands). Molecular-marker-assisted analysis confirmed that the TYLCV resistance offered by Tyking was not controlled by the *Ty-1, Ty-2, Ty-3*, and *Ty-4* genes, and several of these lines were consequently tested with the *Ty-5* CAPS marker, *SlNAC1*. The results showed that the Tyking-derived allele was recessive, so the authors proposed renaming the TY172-derived locus to *ty-5* to better reflect the recessive gene action. Tyking-associated resistance likely corresponds to that of TY172, although further evidence is needed to demonstrate the allelism of the genes (Hutton et al., 2012). In 2015, Lapidot et al. delimited the *ty-5* locus in a single gene encoding the tomato homolog of the messenger RNA surveillance factor Pelo. By analysing a Fla.8638B X Fla.7987 F2 population, Hutton and Scott (2014) confirmed the effect of a *Begomovirus* tolerance gene on chromosome ten, named *Ty-6. Ty-1* is used in breeding programmes worldwide as it confers a high level of tolerance, although it does not show complete resistance, and under conditions of heavy infection, most cultivars end up expressing symptoms (Pérez de Castro, 2007). With molecular-marker-assisted pyramiding, it is possible to obtain plant materials that provide more effective and longer-lasting resistance over time, facilitating the management of traditional and commercial varieties, both outdoors and in greenhouses (Cabrera et al., 2024). Some authors have demonstrated the behaviour of these genes against TYLCV, both individually and in combination (Pérez de Castro et al., 2008; Ozores-Hampton et al., 2013; Prasanna et al., 2014; Scott et al., 2015; Elbaz et al., 2016; Al-Shihi et al., 2018; Wang et al., 2018; Yan et al., 2018; Ren et al., 2022; Ahmed et al., 2023).

In 1998, a breeding programme was launched at the Miguel Hernández University (CIAGRO-UMH) to develop traditional tomato varieties, Muchamiel and De la Pera, with resistance to the three main viruses affecting tomato cultivation (ToMV, TSWV and TYLCV). The goal was to develop pure lines that could be used by farmers to obtain seed throughout each crop cycle or serve as parental lines for the breeding programme. In 2017, introgression of the *ty-5* allele started to complement the previously introduced *Ty-1* resistance.

A priori, this is the first study of TYLCV inoculation under controlled

Table 1
Genotypes of the breeding lines used in this work.

Study 1		Study 2		Study 3	
Muchamiel	De la pera	Muchamiel	De la pera	Muchamiel	De la pera
Ty-1[a]	*ty-5*[a]	*Ty-1*[a]	ss/SS[a]	*Ty-1/ty-5*[a]	*Ty-1/ty-5*[a]
Control ss/SS[c]	ss/SS[a]	ty-5[a]	Control ss/SS[c]	ss/SS[a]	ss/SS[a]
Control *ty-5*[b]	Control ss/SS[c]	Control ss/SS[c]	Control *ty-5*[b]	*ty-5*[a]	*ty-5*[a]
	Control *ty-5*[b]	Control *ty-5*[b]		*Ty-1*[a]	*Ty-1*[a]
				Control *Ty-1*[b]	Control *Ty-1*[b]
				Control *ss/SS*[c]	Control *ss/SS*[c]
				Control *ty-5*[b]	Control *ty-5*[b]

*Control *ty-5*: donor parent (line TX 468-RG on loan from Dr. Rafael Fernández-Muñoz del IHSM La Mayora-CSIC). [a]BC5S1 breeding lines (contain the *Tm-2*^{a} and *Sw-5* alleles in the homozygous state). [b]Breeding line with only *Ty-1* or *ty-5* genes. [c]Traditional cultivars.

Table 2
Symptom severity (mean of the assessed plants) after inoculation of the genotypes of each evaluation according to the scale proposed by Friedmann et al. (1998).

Muchamiel	plants evaluated	mean severity index (0–4)				De la pera	plants evaluated	mean severity index (0–4)			
Genotypes study 1		7 dpi	14 dpi	21 dpi	28 dpi			7 dpi	14 dpi	21 dpi	28 dpi
Ty-1[a]	6	0.00	0.25	0.00	0.00	*ty-5*[a]	12	0.04	0.75	1.17	1.29
Control ss/SS[c]	10	0.60	1.50	3.80	3.90	Control ss/SS[c]	18	0.53	3.39	4.00	4.00
Control *ty-5*[b]	23	0.5	0.35	0.43	0.50	ss/SS[a]	8	0.31	1.63	4.00	3.88
						Control *ty-5*[b]	23	0.50	0.35	0.43	0.50
Genotypes study 2		7 dpi	14 dpi	21 dpi	28 dpi			7 dpi	14 dpi	21 dpi	28 dpi
Ty-1[a]	12	0.00	0.04	0.00	0.00	Control ss/SS[c]	16	0.31	3.38	3.75	3.94
Control ss/SS[c]	19	0.21	3.68	3.95	4.00	ss/SS[a]	18	0.45	3.33	3.68	3.67
ty-5[a]	20	0.00	0.55	0.00	0.00	Control *ty-5*[b]	16	0.00	0.59	0.16	0.34
Control *ty-5*[b]	16	0.00	0.59	0.16	0.34						
Genotypes study 3		-	15 dpi	24 dpi	30 dpi			-	15 dpi	24 dpi	30 dpi
Ty-1/ty-5[a]	10	-	0.00	0.00	0.00	*Ty-1/ty-5*[a]	9	-	0.00	0.00	0.00
ss/SS[a]	10	-	0.15	1.80	4.00	ss/SS[a]	10	-	1.75	2.90	4.00
ty-5[a]	10	-	0.00	0.45	0.40	*ty-5*[a]	8	-	0.25	0.13	0.50
Ty-1[a]	10	-	0.00	0.35	0.20	*Ty*-1[a]	8	-	0.00	0.00	0.13
Control Ty-1[b]	10	-	0.00	0.00	0.30	*Control Ty*-1[b]	9	-	0.00	0.50	0.39
Control ss/SS[c]	8	-	1.06	2.69	4.00	Control ss/SS[c]	10	-	0.95	3.80	4.00
Control *ty-5*[b]	10	-	0.00	0.10	0.35	Control *ty-5*[b]	10	-	0.00	0.10	0.35

dpi: days post-inoculation. *Control *ty-5*: donor parent (line TX 468-RG). [a]BC5S1 breeding lines (containing the *Tm-2*^{a} and *Sw-5* alleles in the homozygous state). [b]Breeding line with only *Ty-1* or *ty-5* genes. [c]Traditional cultivars.

conditions in lines carrying the *Ty-1* and *ty-5* genes. The aim of the study was to evaluate the behaviour of tomato breeding lines carrying these two genes and test their level of tolerance to TYLCV.

2. Materials and methods

2.1. Plant material

Local tomato varieties Muchamiel and De la pera are very popular in the Vega Baja del Segura region in southeastern Spain due to the exceptional organoleptic quality of their fruits. Muchamiel fruits have a melting texture and mild flavour, are large in size (180 g to 300 g), flattened, and strongly ribbed (García-Martínez et al., 2011). De la pera fruits have a juicy and firm texture, a high proportion of seeds and mucilage, and an intense flavour. The weight of the latter ranges from 75 to 125 g, while the shape varies from elongated oval to bell-shaped, with dark green shoulders and no ribs (García-Martínez et al., 2012). In this study, we conducted three experiments (studies 1 and 2 include symptom severity and study 3 includes symptom severity and viral load accumulation) using several Muchamiel and De la pera breeding lines obtained in the CIAGRO-UMH tomato breeding programme. We studied breeding lines with all homozygous combinations of the *Ty-1* and *ty-5* genes. Obtained after five backcrosses and two selfings, these lines also contain the *Tm-2*^{a} and *Sw-5* alleles (confer resistance to tomato mosaic virus (ToMV) and tomato spotted wilt virus (TSWV) respectively) in the homozygous state. Breeding lines with *Ty-1* or *ty-5* genes only and traditional cultivars were used as controls (Table 1). The genotypes of three plants per line were verified using the molecular markers linked to each gene, as described in Carbonell et al. (2018).

2.2. Inoculation

Inoculation was carried out using the agroinoculation method (Kheyr-Pour et al., 1994). The *Agrobacterium tumefaciens* used was transformed with a partial dimeric copy of the TYLCV-IL strain (provided by Dr. Eduardo Rodríguez-Bejarano, University of Málaga). The bacteria were cultivated for 48 h at 26 ºC and in the dark in LB Broth medium, supplemented with kanamycin (100 mg/L). Cells were concentrated ten-fold by centrifugation at 4600 rpm for 20 mins. Inoculation was conducted in plants in the 3 true-leaf stage by injecting the bacterial culture into the axillary buds of the three youngest leaves. Two inoculations with the TYLCV isolate were performed. Plants were grown in pots in a growht chamber with 25 °C temperature, 60 to 65% and 95 to 99% relative humidity (day/night), 34 μEm^{-2}s^{-1} irradiance and 16/8 photoperiod (light/dark).

2.3. Disease assessment

Symptom severity was assessed in each plant individually at 7, 14, 21 and 28 days post-inoculation (dpi) in studies 1 and 2, and at 14, 21 and 28 dpi in study 3. The assessment was based on the scale proposed by Friedmann et al. (1998), where 0 = no symptoms; 1 = very slight symptoms, with yellowing of the leaflet margins on apical leaves; 2 = mild symptoms, with some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping, with some reduction in size, yet the plants continue to grow; and 4 = severe symptoms, with very severe plant stunting and yellowing and pronounced cupping and curling, and the plants cease to grow.

The viral load accumulation was assessed by qPCR to complement and support the results obtained in the previous assays. The same genotypes used in the third symptom severity study were evaluated for both Muchamiel and De la pera. Viral accumulation was quantified by qPCR at 15 and 30 dpi. Total DNA was isolated from the apical leaf of five plants of each genotype using the CTAB method (Doyle and Doyle, 1990). After quantification using a Nanodrop 1000 spectrophotometer (Thermo Scintific, Waltham, MA, USA), each sample was adjusted to a final concentration of 5 ng/μl. The qPCR was carried out in 15-μl reactions, containing 7.5 μl of the FastStart Essential DNA Green Master kit (Roche) mix, 1.5 μl of each primer (10 μM) and 15 ng of the isolated DNA as template. Primers TYLCV-INIA1-F (5′- CCCCCTTTAATTTGAATGGGCTT-3′, positions 1907–1929) and TYLCV-INIA2- R (5′- CATTGATGACGT AGACCCGCA-3, positions 2017–1997) were used for the amplification of viral DNA (Pérez-Padilla et al., 2020). Amplification of the endogenous cytochrome oxidase gene was used as an internal control with the primer pair COX-F (5′- CGTCGCATTCCAGATTATCCA) and COX-R (5′-CAACTACGGATATATAAGAGCCAAAACTG-3′), described by Weller et al. (2020). All reactions were run in a Roche LightCycler 480. The cycling conditions were 95 ºC for 10 min, and 40 cycles at 95 ºC for 15 s, 65 ºC for 30 s and 60 ºC for 60 s. The cycle threshold (Ct) was calculated for the viral DNA and the internal control gene in each sample. Relative accumulation of TYLCV was estimated by the comparative Ct method, using the formula $2^{-\Delta\Delta Ct}$, where ΔΔCt is the difference between the ΔCt of each sample (ΔCtsample: Ctreference genes –Ctvirus) and the ΔCt of the calibrator sample (ΔCtcalibrator). A negative control was used as the

Table 3
p-values of the one-factor GML analyses of variance, with genotypes as a factor. Significance levels: ns ($p > 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$). The mean ($2^{-\Delta\Delta Ct}$) and groups (values followed by the same letter are not significantly different at the 5% level by Fisher) obtained by Fisher's LSD tests are included. The genotypes analysed correspond to study 3.

Varietal type		15 dpi	30 dpi
		GML Test	
Muchamiel	*p*-value	**	***
De la pera	*p*-value	***	***
		Fisher's Multiple Range Test	
Muchamiel	**Genotype**		
	Ty-1/ty-5[a]	912.3 a	2653.6 a
	ss/SS[a]	7190.2 b	14,768.0 c
	ty-5[a]	405.0 a	4369.8 ab
	Ty-1[a]	97.0 a	621.8 a
	Control Ty-1[b]	642.3 a	345.4 a
	Control *ss/SS*[c]	7622.43 b	8048.2 b
	Control *ty-5*[b]	280.2 a	1077.9 a
De la pera	**Genotype**		
	Ty-1/ty-5[a]	790.2 a	302.4 a
	ss/SS[a]	4184.2 a	15,160.8 c
	ty-5[a]	2391.0 a	5255.7 b
	Ty-1[a]	166.8 a	346.2 a
	Control Ty-1[b]	138.0 a	236.5 a
	Control *ss/SS*[c]	11,548.2 b	13,485.4 c
	Control *ty-5*[b]	280.2 a	1077.9 a

dpi: days post-inoculation. *Control *ty-5*: donor parent (line TX 468-RG). [a]BC5S1 breeding lines (containing the *Tm-2*[a] and *Sw-5* alleles in the homozygous state). [b]Breeding line with only *Ty-1* or *ty-5* genes. [c]Traditional cultivars.

calibrator sample in all assays.

2.4. Statistical analysis

Analyses of variance were performed according to the one-factor generalized linear model, with genotypes as a factor. Fisher's Least Significant Difference (LSD) procedure was used for the discrimination of means with a confidence level of 95%. Statgraphics and Excel were used.

3. Results

All plants showed a homogeneous response depending on the genotype studied (Table 2). Susceptible controls (ss/SS[c]) showed severe symptoms, especially from 14 dpi onwards. At this stage, the virus reproduces rapidly and spreads throughout the plant. The tolerant controls (*ty-5*[b] and *Ty-1*[b]) delayed the development of the disease. The symptoms were very mild and remained constant throughout the 28 dpi. The results of the breeding lines studied with all homozygous combinations of the *Ty-1* and *ty-5* genes were consistent with those of the controls. Susceptible genotypes (ss/SS[a]) showed severe symptoms, and resistant genotypes (*Ty-1/ty-5*[a], *ty-5*[a] and *Ty-1*[a]) showed very mild symptoms. The phenotypic responses to TYLCV can be classified into two groups: the susceptible genotypes (ss/SS) for the two genes and the resistant genotypes (*Ty-1, ty-5* and *Ty-1/ty-5*), presenting *Ty-1, ty-5* or both. In all three studies, the susceptible genotypes showed clear symptoms (the average disease severity index (DSI) of the three studies was 3.94) in contrast to the tolerant genotypes (whose average DSI of the three studies was 0.31) (Table 2). The analysis of variance of the viral accumulation quantified by qPCR (included in study 3) showed statistically significant differences in the two varietal types, Muchamiel and De la pera (Table 3). The susceptible genotypes (ss/SS) showed the highest levels of virus accumulation, while the resistant genotypes (*Ty-1, ty-5* and *Ty-1/ty-5*) generally exhibited low levels of virus accumulation (Table 3).

The results obtained indicate that in the three disease incidence studies and in the viral accumulation analysis (included in study 3), the genotypes without *Ty-1* and *ty-5* genes (ss/SS) showed the highest degree of disease severity and viral load. In general, the remaining genotypes for one or both genes showed mild symptoms (Figs. 1, 2 y 3) and a low level of viral accumulation (Fig. 4). Differences were detected between genotypes carrying one or both genes. In studies 1 and 2 (Figs. 1 and 2), the symptoms observed in the phenotype of plants with the *Ty-1* gene (values ranging from 0.04 to 0.25) only appeared at 14 dpi in Muchamiel. These values were slightly lower than those observed in plants with *ty-5* in all cases (Figs. 1 and 2). This difference was most noticeable in the De la pera varietal type in study 1 (Fig. 1), with values ranging from 0.50 to 1.29, while the values of the rest of the genotypes with *ty-5* did not exceed 0.59 points (Figs. 1 and 2). In study 3, a new

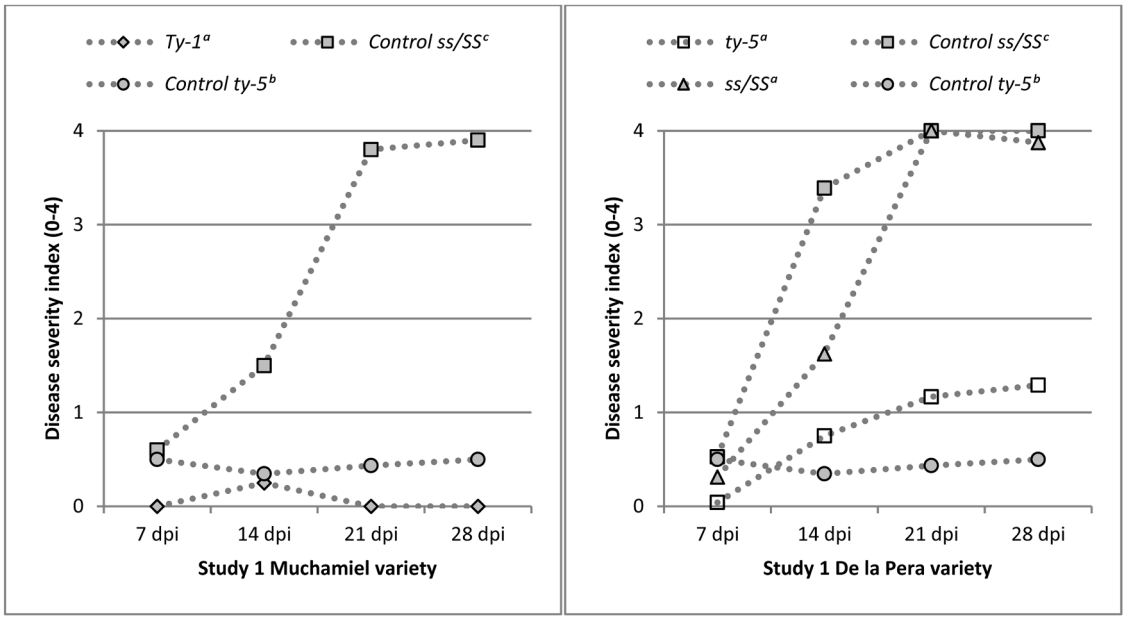

Fig. 1. Symptom severity (mean of plants tested) in study 1 after inoculation according to the scale proposed by Friedmann et al. (1998). dpi: days post-inoculation. *Control *ty-5*: donor parent (line TX 468-RG). [a]BC5S1 breeding lines (containing the *Tm-2*[a] and *Sw-5* alleles in the homozygous state). [b]Breeding line with only *Ty-1* or *ty-5* genes. [c]Traditional cultivars.

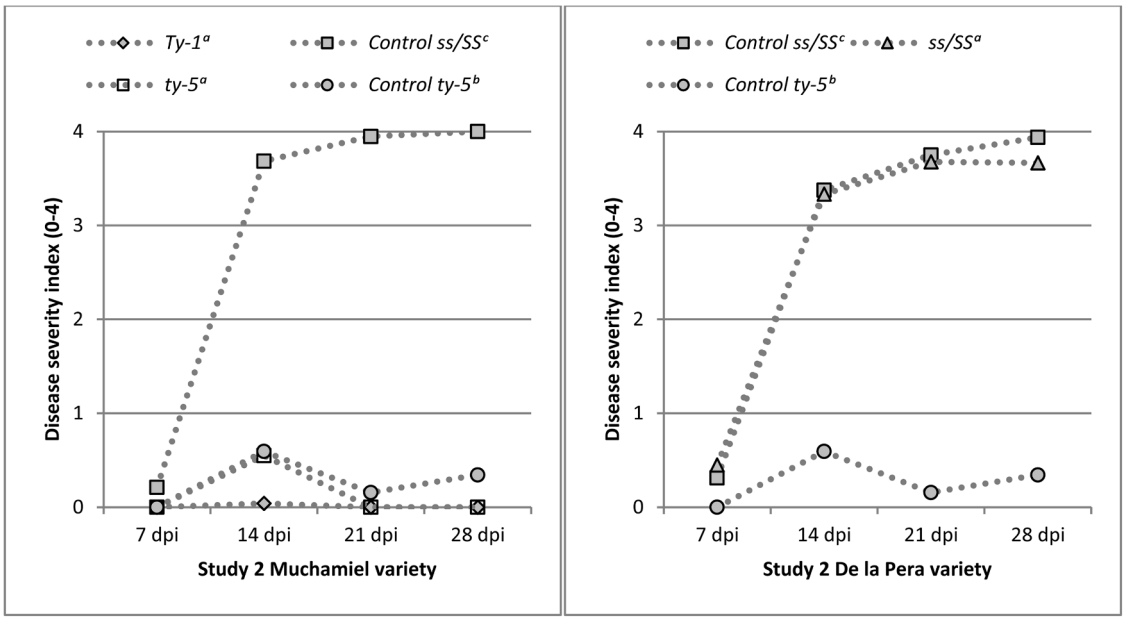

Fig. 2. Symptom severity (mean of plants tested) of study 2 after inoculation according to the scale proposed by Friedmann et al. (1998). dpi: days post-inoculation. *Control *ty-5*: donor parent (line TX 468-RG). [a]BC5S1 breeding lines (containing the *Tm-2*^{a} and *Sw-5* alleles in the homozygous state). [b]Breeding line with only *Ty-1* or *ty-5* genes. [c]Traditional cultivars.

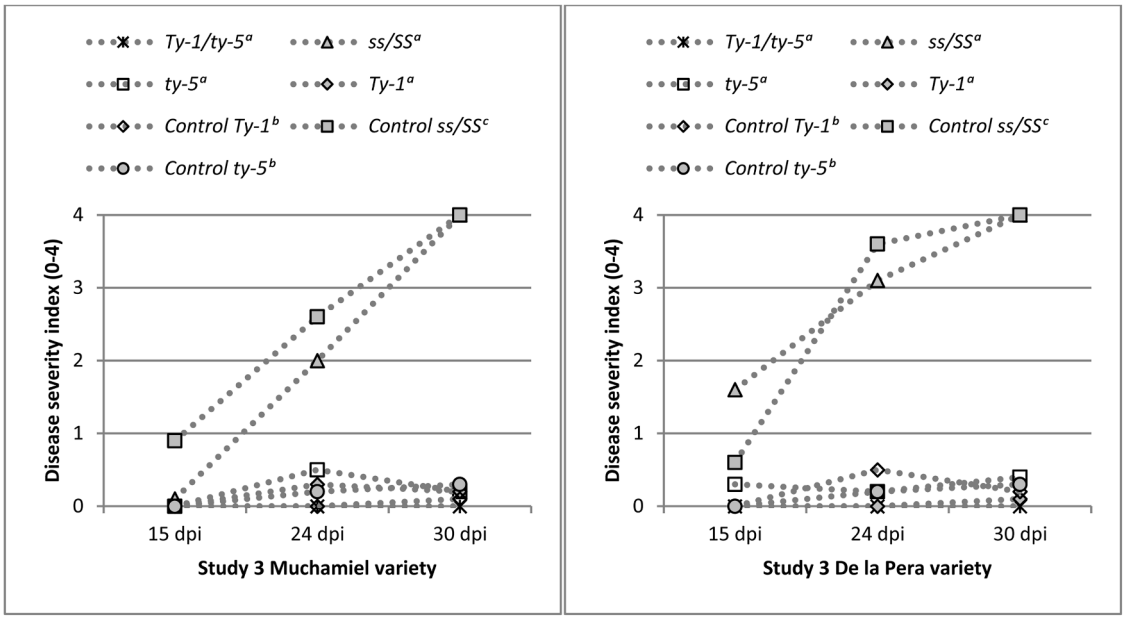

Fig. 3. Symptom severity (mean of plants tested) of study 3 after inoculation according to the scale proposed by Friedmann et al. (1998). dpi: days post-inoculation. *Control *ty-5*: donor parent (line TX 468-RG). [a]BC5S1 breeding lines (containing the *Tm-2*^{a} and *Sw-5* alleles in the homozygous state). [b]Breeding line with only *Ty-1* or *ty-5* genes. [c]Traditional cultivars.

genotype was introduced: the double homozygote for both genes (*Ty-1/ty-5*). The phenotype of this genotype showed no symptoms in any of the varietal types. In the other genotypes, the values were similar to those obtained in studies 1 and 2 (Fig. 3). In the case of viral accumulation, as shown in Fig. 4, two groups were differentiated: the genotypes containing the genes *Ty-1, ty-5* and *Ty-1/ty-5*, and those that did not (ss/SS). The *Ty-1/ty-5* genotype showed a higher viral load than the genotypes with some of the genes in some cases, despite being statistically grouped together. Viral accumulation in the varietal type De la pera at 30 dpi with genotype *ty-5*[a] was higher than accumulation in the rest of the resistant genotypes, but still lower than in the susceptible genotypes (Fig. 4).

4. Discussion

Ty genes have shown different levels of tolerance (Ji et al., 2007, 2009a; Shahid et al., 2013). All *Ty*-loci described have been shown to allow virus replication to varying degrees, which defines them as asymptomatic carriers (Yan et al., 2021). In the present study, mainly during the early stages, some resistant genotypes showed no symptoms, although they did show viral load. Pereira-Carvalho et al. (2015) suggested that using *ty-5* is an effective breeding method for reducing

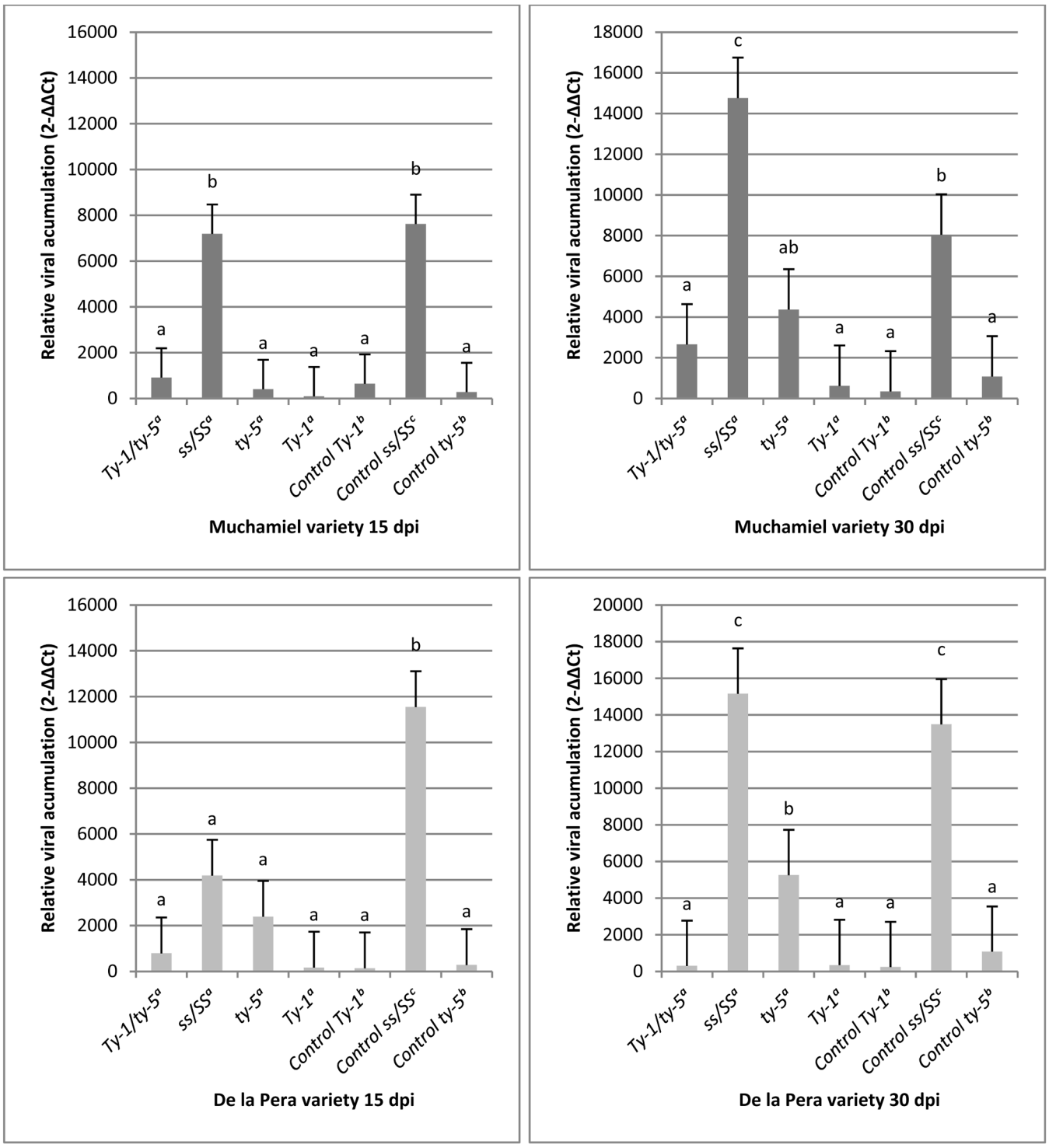

Fig. 4. Relative viral accumulation ($2^{-\Delta\Delta Ct}$) of genotypes in study 3. Different letters indicate statistically significant differences according to Fisher's LSD test. Standard error is included. dpi: days post-inoculation. *Control *ty-5*: donor parent (line TX 468-RG). [a]BC5S1 breeding lines (containing the *Tm-2*^{a} and *Sw-5* alleles in the homozygous state). [b]Breeding line with only *Ty-1* or *ty-5* genes. [c]Traditional cultivars.

TYLCD damage. In a study aimed at understanding the restricted accumulation of an isolate of the TYLCV strain Israel (TYLCV-IL) in TX 468-RG and evaluating how this resistance could help limit the spread of the virus in field conditions, the researchers found that the monogenic recessive resistance to TYLCV-IL in TX 468-RG (the control genotype *ty*-5^b in this study) significantly hindered systemic virus infection. This resulted in a notable reduction in both primary (virus spread to healthy plants from an external source of viruliferous vectors) and secondary (virus spread from virus-infected source plants to healthy plants) virus transmission. In Tunisia, in open field conditions, Elbaz et al. (2016) evaluated a set of tomato entries with different *Ty* combinations (*Ty-1, Ty-1/Ty-2, Ty-1/Ty-3/Ty-2, Ty-2, Ty-2/Ty-3, Ty-2/Ty-3a, ty-2/ty-5 and ty-5*) provided by AVRDC. The trials were conducted in 2013 and 2015 from September to December, when whiteflies are abundant. The goal was to identify the specific genes and gene combinations that effectively reduced the incidence and severity of TYLCV caused by Tunisian

begomoviruses. The *Ty-1/Ty-3* allele provided the highest level of resistance. Entries with *Ty-1* and *Ty-1/Ty-2* were resistant to TYLCD and had similar resistance levels as *Ty-1/Ty-3*. However, the line homozygous for *ty-5* only did not provide high levels of resistance to TYLCD. *Ty-2/Ty-5* showed 32.35% and 43.3% greater reductions in TYLCD incidence and severity than the *Ty-5* entry, and these differences were significant. In another similar study, in an open field and during two growing seasons (September to February 2012–2013 and 2013–2014), the authors evaluated different combinations with the *Ty-1, Ty-2, Ty-3, Ty-3a* and *ty-5* genes. All lines containing the *Ty* genes performed better than susceptible controls, with lower disease incidence, severity and viral DNA load. Symptom severity was highest in lines carrying only *Ty-2*, followed by lines homozygous for *Ty-3a* and *Ty-2*. Lines carrying *ty-5* or *ty-5/Ty-2* remained consistently symptom-free in both years, and their mean viral loads were the lowest, although they were not statistically different from most other lines except those with *Ty-2* only (Al-Shihi et al., 2018).

In the current study, the *Ty-1/ty-5* genotype was the only one with no disease symptoms at any stage, despite showing a viral load. This means that, like in the cases of the other genotypes, there will always be the possibility of whitefly transmission and virus replication, leading to high levels of infection and potentially overcoming resistance. In the present study, the results are quite clear. However, considering the information available in other publications, it is challenging to definitively establish the behaviour of the *Ty-1* and *ty-5* genes due to the variations exhibited by different combinations of these genes, the TYLCV isolate and the varying degrees of infection. These differences may be due to the diversity of TYLCVD-causing Begomoviruses (Al-Shihi et al., 2018) and their ability to overcome resistance (Yan et al. 2021).

The genotypes of the breeding lines studied also contained the *Tm2*^{a} and *Sw-5* alleles, as mentioned in Materials and Methods, Section 2.1. These genotypes could influence the plant response to symptom level and virus load. The results obtained suggest that the introgressed *Tm-2*^{a} and *Sw-5* alleles are not involved in the development of the disease or in the defence mechanism provided by the *Ty-1* and *ty-5* alleles. The behaviour of the genotypes containing *Tm-2*^{a} and *Sw-5* and their counterparts without them was similar in all the studies carried out. In this study, we have demonstrated that both *Ty-1* and *ty-5*, whether used together or individually, provide a similarly high level of resistance. Based on the performance of these lines carrying these genes, they could be used as parental lines in breeding programmes to obtain new cultivars with some added value. This could also lead to the development of lines that allow farmers to save seeds from different landraces for the following growing season. In addition, pyramidalization provides more effective and longer-lasting resistance over time. The virus will encounter more barriers in overcoming resistance and infecting the plant. When the virus exceeds a certain resistance, it can spread rapidly in certain cultivars, so the search for sources of resistance must continue (Yan et al., 2018). *Ty* gene pyramiding appears to be the best option for fighting against TYLCVD. To this end, we began introducing *Ty-2* (Cabrera et al., 2021) in 2021, with the goal of obtaining *Ty-1/ty-5/Ty-2* lines and increasing genetic resistance to TYLCV. The performance of these genotypes will be reported in future publications.

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CRediT authorship contribution statement

José Ángel Cabrera: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pedro Carbonell:** Methodology. **Aranzazu Alonso:** Methodology. **Clara Pérez-Moro:** Methodology. **Ana Pérez de Castro:** Methodology, Writing – review & editing. **Juan José Ruiz:** Conceptualization, Writing – review & editing. **Santiago García-Martínez:** Conceptualization, Data curation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2025.114012.

Data availability

Data will be made available on request.

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