

Reaction of tomato hybrids carrying the *Ty-1* locus to Brazilian bipartite *Begomovirus* species

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ABSTRACT

The number of tomato-infecting begomoviruses has increased in Brazil after the introduction of the polyphagous vector *Bemisia tabaci* biotype B. The *Ty-1* locus, introgressed from *Lycopersicon chilense*, controls tolerance to species of the monopartite *Tomato yellow leaf curl virus* (TYLCV) complex in Europe and the Middle East. However, little information is available about the *Ty-1* effectiveness against species of the bipartite *Begomovirus* complex occurring in Brazil. Heterozygous (*Ty-1/ty-1*) and homozygous (*ty-1/ty-1*) hybrids were evaluated for reaction to *Begomovirus* isolates under open field conditions in two growing areas in Central Brazil. Test plants were evaluated under natural inoculation with high vector pressure. Evaluation was done using a disease assessment scale (DAS) varying from 1= no symptoms to 4= severe symptoms. Systemic infection was evaluated via polymerase chain reaction (PCR) using 'universal' *Begomovirus* primers. In the trial #1, the hybrids (*Ty-1/ty-1*) and (*ty-1/ty-1*) had 35% and 95% of plants with symptoms and 75% and 100% of plants with positive PCR, respectively. In the trial #2, only 20% of the *Ty-1/ty-1* hybrid plants were symptom-free with both hybrids displaying 100% of plants with positive PCR. This reaction of the *Ty-1* hybrid to bipartite *Begomovirus* species was similar to that reported in Europe and the Middle East to the TYLCV complex with a large number of plants being neither virus-free nor symptom-free. On the other hand, symptom expression of the *Ty-1* hybrid was significantly milder than *ty-1/ty-1* hybrids in both trials (DAS = 1.35 vs. 2.70 for the trial #1 and DAS = 2.05 vs. 3.95 for the trial #2). Nucleotide sequencing indicated the presence of isolates genetically related to *Tomato rugose mosaic virus* (ToRMV) in the trial #1 and a mixed infection of ToRMV and Tomato yellow vein streak virus in the trial #2. Therefore, the *Ty-1* locus seems to control a "tolerance" response to distinct *Begomovirus* species. Resistance gene clusters is a common feature in the tomato genome, particularly at the chromosome 6 where *Ty-1* is located. Therefore, additional studies are necessary to confirm if this tolerance to a range of begomoviruses is controlled by *Ty-1* alone or by the action of distinct, closely linked genes.

Keywords: bipartite geminivirus, *Lycopersicon chilense*, resistance, tomato.

RESUMO

Resposta de híbridos de tomateiro contendo o locus *Ty-1* contra espécies brasileiras de *Begomovirus* de genoma bipartido

O número de espécies de *Begomovirus* infectando tomateiro no Brasil aumentou significativamente após a introdução do vetor (*Bemisia tabaci* biótipo B). O locus *Ty-1*, derivado de *Lycopersicon chilense*, controla a tolerância para isolados de *Begomovirus* de genoma monopartido pertencentes ao complexo do *Tomato yellow leaf curl virus* (TYLCV) que ocorre na Europa e Oriente Médio. No entanto, existe escassa informação a respeito da eficiência do locus *Ty-1* contra as diferentes espécies do complexo de *Begomovirus* de genoma bipartido reportadas no Brasil. Híbridos heterozigotos (*Ty-1/ty-1*) e homozigotos (*ty-1/ty-1*) foram avaliados em campo aberto em duas regiões produtoras do Brasil Central. As condições experimentais foram de inóculo natural sob elevada densidade populacional de moscas-brancas virulíferas. A avaliação foi conduzida empregando um índice de severidade de sintomas (ISS) para descrever variando de 1= sem sintomas a 4= sintomas severos. A frequência de infecção sistêmica foi avaliada via polymerase chain reaction (PCR) usando "primers universais" para detecção de espécies de *Begomovirus*. No ensaio 1, os híbridos heterozigotos (*Ty-1/ty-1*) e homozigotos (*ty-1/ty-1*) apresentaram 35% e 95% de plantas exibindo sintomas e 75% e 100% de plantas com PCR positivo para infecção sistêmica, respectivamente. No ensaio 2, observou-se apenas 20% das plantas do híbrido *Ty-1/ty-1* sem sintomas virais, com ambos os híbridos apresentando 100% das plantas com PCR positivo. A resposta do híbrido contendo o locus *Ty-1* foi similar à reportada para os begomovirus do grupo do TYLCV no Continente Europeu e Oriente Médio, tendo sido observada a presença de plantas sintomáticas e com multiplicação sistêmica do vírus. No entanto, em ambos os ensaios, a expressão de sintomas no híbrido heterozigoto foi mais suave que aquela apresentada pelos híbridos suscetíveis (ISS = 1,35 vs. 2,70 no ensaio 1 e ISS = 2,05 vs. 3,95, no ensaio 2). Análise da sequência do DNA viral indicou a presença de isolados geneticamente relacionados à espécie *Tomato rugose mosaic virus* (ToRMV) no ensaio 2 e uma infecção mista de ToRMV e Tomato yellow vein streak virus (ToYVSV) no ensaio 2. Estes dados indicam que o locus *Ty-1* confere uma reação do tipo "tolerância" contra distintas espécies de *Begomovirus*. Estreita ligação de genes de resistência e/ou tolerância a doenças é uma característica do genoma do tomateiro, especialmente no cromossomo 6 onde o locus *Ty-1* está posicionado. Desta forma, estudos adicionais são necessários para demonstrar que a tolerância a distintos *Begomovirus* bipartidos é de fato conferida exclusivamente pelo locus *Ty-1* ou pela presença de outros genes ligados.

Palavras-chave: geminivirus bipartido, *Lycopersicon chilense*, resistência, tomate.

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Severe yield losses of tomatoes (*Lycopersicon esculentum* Mill.) caused by infection of viral species from

the genus *Begomovirus* (Family: *Geminiviridae*) became a recurrent problem throughout the Mediterranean

region, the Middle East, Africa and Southeast Asia (Czosnek & Laterrot, 1997; Moriones & Navas-Castillo,

2000). Begomoviruses are also present in the tomato-producing regions in the New World including North America, Caribbean area, Central and South America (Polston & Anderson, 1997; Faria *et al.*, 2000).

The viruses predominant in the Old World area are vectored by distinct whitefly (*Bemisia tabaci*) biotypes and these viruses belong to a group of closely related monopartite species collectively named as the Tomato yellow leaf curl virus (TYLCV) complex (Moriones & Navas-Castillo, 2000). In Brazil, the variability of the tomato-infecting begomoviruses increased significantly after the introduction of *B. tabaci* biotype B (= *B. argentifolii*) in the early 1990's (Ribeiro *et al.*, 1994; França *et al.*, 1996). In comparison with the situation in the Old World, the variability of the tomato-infecting begomoviruses in Brazil seems to be higher as revealed by nationwide surveys in which at least eight provisional new *Begomovirus* species have been reported on tomatoes (Faria *et al.*, 2000; Ribeiro *et al.*, 2003). All the surveys conducted in Brazil indicated the exclusive presence of begomoviruses with bipartite genomes with an apparent absence of monopartite species (Ribeiro *et al.*, 2003; Inoue-Nagata *et al.*, 2004).

Considerable effort has been made to identify resistance genes to begomoviruses through extensive screening of germplasm from cultivated and wild *Lycopersicon* species. Such screening programs indicated that accessions of the wild species *L. chilense* Dunal are among the best sources of resistance to begomoviruses (Zakay *et al.*, 1991; Michelson *et al.*, 1994; Giordano *et al.*, 1999; Griffiths & Scott, 2001; Santana *et al.*, 2001). One TYLCV resistance locus from *L. chilense* 'LA 1969' was characterized as being partially dominant and was named as *Ty-1* (Michelson *et al.*, 1994). The reaction of the *Ty-1* locus-carrying lines to TYLCV isolates has been described as "tolerance" because the plants became infected (with detectable levels of viral DNA) but displayed attenuated symptom expression. Efforts were made to introgress this locus into *L.*

esculentum cultivars (Zamir *et al.*, 1994) and it became one of the most reliable sources of resistance for tomato breeding programs in Europe and Israel. The dominant nature of the *Ty-1* locus allows it to be directly employed for the development of TYLCV tolerant hybrids. A tight linkage of the *Ty-1* locus with susceptibility to root-knot nematodes (*Meloidogyne* spp.) was observed during the development of TYLCV tolerant inbred lines. Additional studies indicated that the genetic location of *Ty-1* in the tomato map is at the top of the chromosome 6 in close repulsion phase linkage to the *Mi* (root-knot nematode resistance) gene (Zamir *et al.*, 1994; Pan *et al.*, 2000).

Recent reports have indicated good levels of tolerance of *Ty-1* locus-carrying lines to the bipartite *Tomato mottle virus* (TMoV) in Florida (Scott *et al.*, 1996) and to Brazilian bipartite *Begomovirus* isolates (Santana *et al.*, 2001; Matos *et al.*, 2003). However, there is a very little of information about the effectiveness of the *Ty-1* locus on individual bipartite *Begomovirus* species occurring in Brazil. The objective of the present work was to evaluate the levels and spectrum of field tolerance of a hybrid carrying the *Ty-1* locus against species belonging to the Brazilian bipartite *Begomovirus* complex.

MATERIAL AND METHODS

One experimental hybrid heterozygous for the *Ty-1* locus and two susceptible (*ty-1/ty-1*) hybrids were evaluated for their reaction to *Begomovirus* in two field trials conducted under natural inoculation with high vector pressure. In the trial #1, the experimental hybrid (*Ty-1/ty-1*) was cultivated together with the susceptible (*ty-1/ty-1*) hybrid 'Carmen' (Sakata Seed Sudamerica) in Goianópolis, Goiás State, Brazil. In the trial #2 the same experimental hybrid (*Ty-1/ty-1*) was cultivated together with the susceptible hybrid 'Débora Plus' (Sakata Seed Sudamerica) in Leopoldo Bulhões, Goiás State, Brazil. Field evaluations were conducted from February to June (2003) employing

standard pest and disease control spray schemes. The experimental design was a complete randomized design with four replications with five plants per plot. Trial #1 was drip-irrigated while trial #2 was furrow-irrigated. Individual plants were visually evaluated for their reaction to *Begomovirus* using a disease assessment scale (DAS) where 1= no conspicuous symptoms; 2= mild mottling, chlorosis and/or mosaic and restricted to the apical part of the plant; 3= intermediate chlorosis and/or mosaic with the plants showing some levels of growth reduction and curling; and 4= overall chlorosis, severe stunting (Giordano *et al.*, 2005). The trait percentage of plants with symptoms was estimated by counting the number of individual plants with DAS varying from 2 to 4. Plants rated with DAS =1 were classified as symptom-free. Evaluations were made 60-70 days after transplanting.

Apical leaf samples of individual plants of each plot in both trials were collected in order to check for systemic *Begomovirus* infection. Total DNA was extracted using a modified 2X CTAB buffer and additional purification steps with organic solvents (Boiteux *et al.*, 1999). A segment of the virus DNA-A component was amplified via polymerase chain reaction (PCR) using degenerated primers (PAL1v1978 and PARc496) that have been employed for universal detection of species within the genus *Begomovirus* (Rojas *et al.*, 1993). PCR amplification products were analyzed by agarose-gel electrophoresis stained with ethidium bromide. Samples with typical *Begomovirus* PCR amplicon in the gel analyses were scored as positive for systemic infection. Four infected leaf samples showing *Begomovirus* symptoms were randomly selected in both experimental fields and were used for sequence analysis to determine the corresponding viral species present in the field trials. Sequencing reaction was performed using the BigDye® version 3 (Applied Biosystems) protocol having as template 2µl of the PCR mix containing the amplified *Begomovirus* genomic segment. DNA sequence was analyzed in an Applied Biosystems 3100

Table 1. Field performance of one experimental tomato hybrid (EH) heterozygous and two tomato hybrids without the *Begomovirus* tolerance locus *Ty-1* under natural epidemics of two bipartite viral species at two areas in Goiás State (GO), Brazil. Epidemics in Goianópolis (Trial #1) was caused by the mixture of *Tomato rugose mosaic virus* (ToRMV) and Tomato yellow vein streak virus (ToYVSV) and epidemics in Leopoldo Bulhões (Trial #2) was caused by ToRMV. Brasília, Embrapa Hortaliças, 2005.

Parameters employed for evaluation	Trial # 1 Goianópolis (GO) (ToYVSV and ToRMV)		Trial # 2 Leopoldo Bulhões (GO) (ToRMV)	
	'Carmen' <i>ty-1/ty-1</i>	'EH' <i>Ty-1/ty-1</i>	'Débora P' <i>ty-1/ty-1</i>	'EH' <i>Ty-1/ty-1</i>
	Disease Score*	2.70 A***	1.35 B	3.95 A
% plants with symptoms**	95.00 A	35.00 B	100.00 A	80.00 B
% plants with positive PCR	100.00 A	75.00 B	100.00 A	100.00 A
Fruit yield (t/1000 plants)	4.40	3.96	2.20	4.40

*Disease assessment scale (DAS) where 1= no conspicuous symptoms; 2= mild mottling, chlorosis and/or mosaic and restricted to the apical part of the plant; 3= intermediate chlorosis and/or mosaic with the plants showing some levels of growth reduction and curling; and 4= overall chlorosis, severe stunting.

**% of plants with disease assessment scale (DAS) ranging from 2 to 4.

***Means with different letter in the same line (in each assay) are not different according to t-test (5%).

sequencer at the Genomic Analysis Laboratory (Embrapa Hortaliças). The sequences were compared with GenBank data base using the algorithm BlastN.

A statistical analysis using t-test was employed to compare the means of disease score; percentage of plants with symptoms and percentage of plants with positive PCR. Percentage of plants with symptoms was transformed to arcsen ($x/100$)^{1/2}. Data with values equal to 100 were transformed to $(100-1/4n)$ before transformation, where n corresponds to the number of plants used to estimate the percentage of plants without symptoms. The transformation $X^{1/2}$ was done for the data set related to the percentage of plants with positive PCR. For useful comparisons, the commercial fruit yield (transformed to Metric tons per 1,000 plants) was assessed at the growing season end in both trials, but they were not statistically analyzed.

RESULTS AND DISCUSSION

In the trial #1, the hybrids (*Ty-1/ty-1*) and (*ty-1/ty-1*) had 35% and 95% of plants with conspicuous *Begomovirus*-like symptoms and 75% and 100% of plants with positive PCR, respectively. In the trial #2, only 20% of the hybrid *Ty-1/ty-1* were symptom-free with both hybrids displaying 100% of plants with

positive PCR (Table 1). In both trials, symptom expression of the hybrid with the *Ty-1* locus was significantly more attenuated (milder) than that of the susceptible hybrids (DAS = 1.35 vs. 2.70 for the trial #1 and DAS = 2.05 vs. 3.95 for the trial #2). Fruit yield of the *Ty-1* hybrid was 100% higher than the susceptible hybrid under severe epidemic conditions in the trial #2. However, fruit yield of both hybrids was almost identical in the trial #1.

New tomato-infecting begomoviruses have been reported in Brazil since the introduction of a new *B. tabaci* biotype. Genetic diversity of tomato-infecting begomoviruses have been evaluated via sequence comparisons and phylogenetic analyses. At least eight provisional new *Begomovirus* species were found (Ribeiro *et al.*, 2003). These begomoviruses were initially detected by using 'universal' PCR primers able to amplify conserved fragments of *Begomovirus* DNA-A and/or DNA-B molecules (Rojas *et al.*, 1993). Here we employed a similar strategy using total DNA extracted from tomato leaves sampled in both trials. In all cases we obtained a single PCR amplicon of approximately 1300bp corresponding to a segment of the viral DNA-A molecule. Gel analysis of the PCR products obtained from DNA extracted from the leaf samples of the upper (apical) leaves

showed the presence of a single virus amplicon (i.e. positive reaction for systemic infection) in 75% of the plants of the hybrid with the *Ty-1* locus and 100% in the susceptible (*ty-1/ty-1*) hybrid in the trial #1, whereas 100% of the plants of both resistant and susceptible hybrids were tested positive in the trial #2. Therefore, the reaction of the *Ty-1* hybrid in our experiment was similar to that reported in Europe and the Middle East to TYLCV where a significant number of plants was neither virus-free nor symptom-free (Rom *et al.*, 1993, Lapidot *et al.*, 1997). A sub-set of these PCR amplicons was selected and sequenced in both 5' and 3' directions. The sequence analyses of the viral isolates occurring in trial #1 revealed the presence of a mixed infection with the presence of isolates genetically related to *Tomato rugose mosaic virus* (GenBank accession AF291705) and Tomato yellow vein streak virus (GenBank accession U79998). The sequence analyses of the isolates obtained in trial #2 indicated the exclusive presence isolates related to *Tomato rugose mosaic virus*.

It has been common to hear from Brazilian tomato growers that they prefer *Begomovirus*-susceptible hybrids rather than the currently available tolerant cultivars carrying the *Ty-1* locus. They claim that fruit yield of the susceptible cultivars is sometimes higher even under high incidence of begomoviruses. Our results reinforce the notion that this observation might be correct in specific situations. Differences in fruit yield could result from a great amount of factors related to cultivar performance to management practices, however, it is now well known that infection caused by bipartite begomoviruses can impart significant losses (Giordano *et al.*, 2004).

The already characterized partial dominant nature of the *Ty-1* suggest that tolerance levels to *Begomovirus* can be increased in tomato hybrids homozygous for this locus. However, homozygous *Ty-1* hybrids have a strong limitation due to the undesirable repulsion phase linkage with root-knot nematode resistance gene *Mi* (Zamir *et al.*, 1994, Pan *et al.*, 2000). Tropical and subtropical soils are often infested with

root-knot nematodes (*Meloidogyne* species) and employment of homozygous *Ty-1* hybrids may result in losses due to infection by this group of soil-borne pathogens. Therefore, homozygous hybrids for the *Begomovirus* tolerance *Ty-1* locus are not expected to become prevalent in Brazil, unless this linkage can be broken.

Ty-1-carrying hybrids displayed high tolerance levels to Tomato yellow vein streak virus isolates in field trials conducted in São Paulo State, Brazil (Matos *et al.*, 2003). In addition, nucleotide sequencing of viral isolates showed the presence of different species in the trials #1 and #2. Altogether, these results are indicating that plants carrying the *Ty-1* locus might have a broad-spectrum tolerance to tomato-infecting begomoviruses. Clustering of resistance genes is a common feature in the tomato genome, particularly at chromosome 6 where *Ty-1* is located. Additional studies are thus necessary to confirm if the tolerance to more than one bipartite *Begomovirus* species is controlled by a single gene or a by the action of distinct, closely linked gene(s) at the locus *Ty-1*.

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