CHARACTERIZATION OF THE RESISTANCE OF TOMATO ACCESSIONS FROM THE BGH-UFV TO THE GEMINIVÍRUS *Tomato yellow spot virus*[1]

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ABSTRACT – The viruses transmitted by whiteflies are among those causing relevant losses in tomato cultivation. Among the measures to control these agents, introducing genes for resistance constitutes the main control measure, together with vector control. The objective of this work was to screen for sources of natural resistance to *Tomato yellow spot virus* (ToYSV) in *S. lycopersicum* germplasm from the Banco de Germoplasma de Hortaliças (BGH) of the Universidade Federal de Viçosa (UFV), Minas Gerais, Brazil. The 99 accessions and two susceptible controls were inoculated using biolistics. Inoculated plants were grown under greenhouse conditions. The percentage of plants displaying virus symptoms was evaluated at 10, 20 and 30 days after inoculation (DAI). Virus presence or absence in the inoculated plants was confirmed by hybridization with probes labeled with á-[32P]-dCTP, for each evaluation date. Inoculated plants produced typical disease symptoms showing different behavior on the genotypes in relation to ToYSV. Some of the evaluated genotypes showed higher virus tolerance compared to two susceptible controls, in particular the accessions BGH-2039V and BGH-2041 which showed no symptoms and no viral DNA accumulation in 80% of the inoculated plants at 30 DAI. The results suggest that the selected tomato accesses are good sources of resistance to new tomato cultivars tolerant to ToYSV.

Key Words: Natural resistance, begomovirus, germplasm bank

CARACTERIZAÇÃO DA RESISTÊNCIA DE ACESSOS DE TOMATE DO BGH-UFV PARA O GEMINIVÍRUS TOMATO YELLOW SPOT VIRUS

RESUMO – Os vírus transmitidos por moscas brancas estão entre aqueles que causam prejuízos relevantes no cultivo de tomate. Entre as medidas para controlar esses agentes, introdução de genes para resistência constitui a medida de controle principal, junto com o controle do vetor. O objetivo deste trabalho foi de avaliar fontes de resistência natural ao vírus da mancha amarela de tomate (ToYSV) em germoplasma de S. lycopersicum *do Banco de Germoplasma de Hortaliças (BGH) da Universidade Federal de Viçosa (UFV), Minas Gerais, Brasil. Um total de 99 acessos e dois controles sensíveis foram inoculados usando biolística. Plantas inoculadas foram cultivadas sob condições de estufa. A percentagem de plantas exibindo sintomas de vírus foi avaliada em 10, 20 e 30 dias após a inoculação (DAI). Presença ou ausência de vírus nas plantas inoculadas foi confirmada por hibridização com sondas rotuladas com á-[32P]-dCTP, para cada data de avaliação. Plantas inoculadas produziram sintomas da doença típicos mostrando comportamento diferente sobre os genótipos em relação ao ToYSV. Alguns dos genótipos avaliados mostraram maior tolerância ao vírus em comparação a dois controles sensíveis, em particular as adesões BGH-2039V e BGH-2041 que não mostraram sintomas e nenhum acúmulo de DNA viral em 80% das plantas inoculadas em 30 DAI. Os resultados sugerem que os acessos de tomate selecionados são boas fontes de resistência para novas cultivares de tomate tolerantes ao ToYSV.*

Palavras-chave: Resistência natural, begomovírus, banco de germoplasma.

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1. INTRODUCTION

In Brazil, the tomato crop has a social and economic importance, being cultivated by low and high-technology farmers. The whitefly *Bemisia tabaci* is considered as one of the most important pests in this crop, not only due to the direct damage caused by its feeding, but also because of the transmission of geminiviruses. Since 1994, geminiviruses have become widespread in Brazil, after the detection of the biotype B of the whitefly.

The biotype B has been responsible for virus transmission from wild hosts to tomato. In Minas Gerais state, Ambrozevicius et al. (2002) isolated *Tomato yellow spot virus* (ToYSV) in tomato, and Calegario et al. (2007) characterized its biological properties, emphasizing the precocity of its damage. The possibility of pseudorecombinant formation among ToYSV and other geminiviruses was also demonstrated (Andrade et al., 2006). The high degree of geminivirus species diversity suggests that Brazil is a center of origin and genetic diversity of these pathogens (Fernandes et al., 2008).

For geminiviruses, the use of resistant or tolerant cultivars is the most promising control method compared to the control of weeds, use of virus-free seedlings and chemical control of the whitefly vector.

The feasibility of genetic control through resistant cultivars depends partly on the use of genetic resources containing genes responsible for resistance expression. However, one of the consequences of modern agriculture has been a loss of traditional varieties without a prior knowledge of their genetic potential, a consequence of the need for more homogeneous cultivars with high yield potential.

Thus, the characterization and evaluation of accessions from germplasm banks helps the bank curators to better use the genetic resources in breeding programs, with the possibility of using accessions that contains genes confering resistance to geminiviruses and other pathogens. According to Karp (2002), the characterization of germplasm has been based mainly on morphological descriptors and, less frequently, on agronomical traits.

The Banco de Germoplasma de Hortaliças (BGH), belonging to Universidade Federal de Viçosa (UFV), established in the 1960s, currently has 6.559 accessions from 25 families and 106 species. This germplasm is incorporated into breeding programs to obtain improved varieties (Silva et al., 2001).

The characterization of tomato accessions from the BGH is currently ongoing. Initially the agronomical and morphological characterization of approximately 350 tomato accessions of the BGH was performed. After that, resistance to potyviruses (*Zucchini yellow mosaic virus*, ZYMV and *Pepper yellow mosaic virus*, PepYMV) (Moura et al., 2005; Juhász et al., 2008), whitefly (*Bemisia tabaci* biotype B) (Fernandes et al., 2009), *Tuta absoluta* (Moreira et al., 2005; Oliveira et al., 2009), Phytophthora infestans (Abreu et al., 2008) and geminivirus (*Tomato yellow spot virus*, ToYSV) (Aguilera et al., 2008) was evaluated. Molecular characterization of the accessions (Aguilera et al., 2011) has also been performed as a complement. All the information obtained from these studies is available at the germplasm bank website (http://www.bgh.ufv.br) to support breeding programs.

Our objective in this study was to evaluate the response of 99 accessions of tomato (*Solanum lycopersicum* Mill.) after inoculation with ToYSV under greenhouse conditions.

2. MATERIALS AND METHODS

The experiments were performed in a greenhouse at the Departamento de Fitopatologia/BIOAGRO, Universidade Federal de Viçosa (UFV). A randomized complete design was used. Ninety-nine tomato accessions belonging to the BGH-UFV collection were tested, in addition to two susceptible controls: the F1 hybrid 'Debora' and the cultivar 'Santa Clara'. The seedlings were inoculated with the pToYSV-A1.2 and pToYSV-B1.2 infectious clones (Andrade et al., 2006). Plants were sap-inoculated or biolistically-inoculated (Aragão et al., 1996), using 2 ìg of DNA corresponding to each genomic component. After inoculation, the plants were transferred to 1 L pots and kept in the greenhouse for symptom evaluation.

The presence or absence of the virus was assessed visually, and classified as positive if the observed plant showed at least one of the symptoms characteristic of ToYSV, including mosaic, epinasty, chlorotic spots and leaf curling. The evaluation was performed three times, at 10, 20 and 30 days after inoculation (DAI), quantifying the number of susceptible plants in relation to the total of plants inoculated and the latent period

of the virus measured as the numbers of days between inoculation and the appearance of symptoms.

Molecular hybridization, as described by Gilberson et al. (1991), was used to confirm the viral infection in the experiment. The membranes were submitted to hybridization with a ToYSV-specific probe labeled with á-[32P]-dCTP using the Prime-it II kit (Stratagene), according to the manufacturer's instructions. The hybridization and washes were conducted at high stringency conditions.

The phenotyping of accessions was conducted according to the scale proposed by Tripathi & Varma (2003), with modifications. Based on the percentage of infected plants (number of plants positive by hybridization in relation to the total of plants tested), the accessions were divided into five categories: highly resistant (HR) (0-10%), resistant (R) (11-20%), moderately resistant (MR) (21-40%), susceptible (S) (41-60%) and highly susceptible (HS) (61-100%).

3. RESULTS AND DISCUSSION

The data shown in Table 1 presents the results of virus infectivity in the 99 accessions tested. The latent period, the results of visual assessment and hybridization, as well as the genotyping of accessions at 30 DAI, are shown in this table. The data reveals that, as expected, most of the accessions were susceptible, with 60.61% HS and 22.22% S (82.83% of the total accessions). From the remaining accessions, 15.15% were classified as MR and two accessions, representing 2.02% of the total accessions, were classified as R, with only one of the five tested plants containing viral DNA.

Three types of responses were observed in the accessions: 1) resistance – no symptoms / absence of viral DNA; 2) tolerance – no symptoms / presence of detectable amounts of viral DNA and 3) susceptibility – plants with symptoms / detectable amounts of viral DNA. Similar results were obtained when different sources of resistance were screened for TYLCV (Zakay et al., 1991).

The latent period (LP), defined as the time between inoculation and the appearance of the first symptoms, showed great variability but was correlated with the resistance phenotype. The maximum LP was 25.8 days for accession BGH-2039V, classified as R, and the minimum LP was 4.2 days for BGH-2032, classified as HS with 100% of the plants infected by ToYSV. On average the LP was 14.73 days.

Viral detection by molecular hybridization confirmed the visual assessment carried out at the three evaluation dates. Although it was not performed quantitatively, we could observe a correlation between signal intensity and the resistance phenotype, coinciding with Matos et al. (2003), who found variations in virus accumulation depending on the response of the material evaluated.

Differences in the reaction among plants of the same accession may be explained by the fact that germplasm bank accessions are actually landraces (a mixture of inbred lines), with high variability (Silva et al., 2001). Zakay et al. (1991) characterized *S. lycopersicum* accessions in terms of their resistance to TYLCV, and concluded that a major difficulty in selecting sources of resistance from germplasm banks are the variations in the accessions, leading to variations that can be expressed as different degrees of disease severity. In the same study, the authors demonstrated that symptoms in wild species are generally much weaker than in the cultivated tomato. The symptoms expressed in the inoculated plants showed the presence of the virus and the effectiveness of the method of inoculation employed.

4. CONCLUSION

This study demonstrated that the evaluation method used was effective in differentiating the diversity expressed by *Solanum lycopersicum* accessions when inoculated with ToYSV, characterized by different levels of resistance and susceptibility. The study identified two accessions as the best sources of resistance among the materials evaluated. The high number of susceptible accessions is a measure of the importance of the characterization of 800 accessions belonging to the collection of the Banco de Germoplasma de Hortaliças (BGH) of Universidade Federal de Viçosa (UFV) in finding new sources of resistance to geminiviruses.

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Table 1 - Results of biolistic inoculation of *Tomato yellow spot virus* onto 99 tomato accessions from the BGH-UFV.

Accessions	LP [a](days)	Evaluation [b]		Percent(%)	PH	Accessions	LP(days)	Evaluation		Percent(%)	PH
		V	H					V	H		
BGH-700	6.6	5/5	5/5	100	HS	BGH-2086	24	2/5	3/5	60	S
BGH-2000	5.4	5/5	5/5	100	HS	BGH-2087	25.8	1/5	2/5	40	MR
BGH-2002	4.6	5/5	5/5	100	HS	BGH-2088	15.6	5/5	3/5	60	S
BGH-2003	12.8	4/5	4/5	80	HS	BGH-2089	17	4/5	3/5	60	S
BGH-2004	6.6	5/5	5/5	100	HS	BGH-2091	18.6	3/5	3/5	60	S
BGH-2006	20.6	2/5	5/5	100	HS	BGH-2092	19.8	3/5	2/5	40	MR
BGH-2008	11	4/5	5/5	100	HS	BGH-2093	17.8	3/5	4/5	80	HS
BGH-2013	8.6	5/5	5/5	100	HS	BGH-2095	24.2	1/5	3/5	60	S
BGH-2014	15.8	3/5	4/5	80	HS	BGH-2096	16.8	3/5	2/5	40	MR
BGH-2016	14.8	3/5	5/5	100	HS	BGH-2097	24.4	3/5	3/5	60	S
BGH-2017	11.2	4/5	5/5	100	HS	BGH-2098	18.2	3/5	3/5	60	S
BGH-2018	5.6	5/5	5/5	100	HS	BGH-2100	16.4	3/5	4/5	80	HS
BGH-2019	15	3/5	2/5	40	MR	BGH-2102	22.2	3/5	3/5	60	S
BGH-2020	15.4	3/5	4/5	80	HS	BGH-2105	18.6	3/5	3/5	60	S
BGH-2021	11.8	4/5	3/5	60	S	BGH-2109	9.6	5/5	4/5	80	HS
BGH-2026	6.4	5/5	5/5	100	HS	BGH-2110	17.2	4/5	5/5	100	HS
BGH-2027	5.2	5/5	5/5	100	HS	BGH-2111	15.2	4/5	4/5	80	HS
BGH-2029	10.4	4/5	4/5	80	HS	BGH-2115	14.8	4/5	4/5	80	HS
BGH-2032	4.2	5/5	5/5	100	HS	BGH-2116	9.8	5/5	5/5	100	HS
BGH-2033	16.2	4/5	5/5	100	HS	BGH-2117	10.4	4/5	4/5	80	HS
BGH-2034	18.4	3/5	4/5	80	HS	BGH-2118	16.2	3/5	4/5	80	HS
BGH-2035	17.2	3/5	5/5	100	HS	BGH-2120	5	5/5	4/5	80	HS
BGH-2038	7	5/5	5/5	100	HS	BGH-2121	10.4	4/5	5/5	100	HS
BGH-2039A	16	3/5	5/5	100	HS	BGH-2122	11.4	5/5	5/5	100	HS
BGH-2039V	25.8	1/5	1/5	20	R	BGH-2124	19.8	3/5	3/5	60	S
BGH-2041	25.2	1/5	1/5	20	R	BGH-2125	5	5/5	5/5	100	HS
BGH-2046	7.6	5/5	4/5	80	HS	BGH-2127	10.6	5/5	5/5	100	HS
BGH-2048	11	4/5	4/5	80	HS	BGH-2128	11.8	4/5	5/5	100	HS
BGH-2052	19.6	4/5	4/5	80	HS	BGH-2131	8.6	5/5	3/5	60	S
BGH-2054	7	5/5	5/5	100	HS	BGH-2132	8.4	5/5	5/5	100	HS
BGH-2055	14.8	5/5	5/5	100	HS	BGH-2133	10.4	4/5	5/5	100	HS
BGH-2057	18	4/5	5/5	100	HS	BGH-2134	20.8	3/5	3/5	60	S
BGH-2060	15.4	4/5	4/5	80	HS	BGH-2135	15.4	4/5	3/5	60	S
BGH-2062	16.6	4/5	5/5	100	HS	BGH-2138	17.8	4/5	3/5	60	S
BGH-2064	16	4/5	3/5	60	S	BGH-2141	9.8	4/5	5/5	100	HS
BGH-2065	24.75	1/4	2/4	50	S	BGH-2114	12	5/5	4/5	80	HS
BGH-2068	24.2	2/5	2/5	40	MR	BGH-2143	10	5/5	2/5	40	MR
BGH-2069	13.4	4/5	5/5	100	HS	BGH-4310	14	4/5	2/5	40	MR
BGH-2071	18.8	3/5	2/5	40	MR	BGH-4349	12	4/5	3/5	60	S
BGH-2072	16	3/5	2/5	40	MR	BGH-4350	16.4	4/5	2/5	40	MR
BGH-2073	21.4	2/5	3/5	60	S	BGH-4474	15.6	3/5	5/5	100	HS
BGH-2074	24.2	2/5	4/5	80	HS	BGH-4512	9.8	5/5	5/5	100	HS
BGH-2075	16	5/5	5/5	100	HS	BGH-4541	15	5/5	5/5	100	HS
BGH-2076	23	2/5	2/5	40	MR	BGH-4544	6.8	5/5	5/5	100	HS
BGH-2077	20.6	3/5	3/5	60	S	BGH-4546	7	5/5	5/5	100	HS
BGH-2078	18.8	4/5	2/5	40	MR	BGH-4547	10.8	5/5	3/5	60	S
BGH-2080	23.4	2/5	2/5	40	MR	BGH-4577	10.4	5/5	5/5	100	HS
BGH-2081	21.4	2/5	2/5	40	MR	BGH-4596	20.6	3/5	5/5	100	HS
BGH-2082	20.8	2/5	2/5	40	MR	BGH-4619	16.6	4/5	5/5	100	HS
BGH-2083	13	4/5	3/5	60	S						
Débora	16.09	7/11	7/11	63.64	HS	Santa Clara	13.33	9/12	9/12	75,00	HS

[a]: latent period; [b]: Evaluation V- visual and H-hybridization; [c]: Phenotyping according to the scale proposed by Tripathi & Varma (2003)

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