Control failures of Fusarium wilt on tomatoes and resistance of cultivars to the three races of the pathogen[1]

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ABSTRACT

The use of resistant cultivars is the main strategy to control Fusarium wilt on tomato, caused by *Fusarium oxysporum* f. sp. *lycopersici*. This study aimed to quantify the incidence and distribution of this disease in the region of Nova Friburgo, RJ, as well as the type of resistance of tomato cultivars to the three FOL races. Samples of healthy and wilted plants from 40 properties were evaluated for the presence of vascular discouloration. Seventeen tomato cultivars were evaluated for resistance to the three FOL races using a grading scale. Classification for resistance/susceptibility, incidence and percentage of infection of the vascular system, fresh weight accumulation and seedling length were determined. All the cultivars analysed behaved as similar to immune (SI) to race 1 of FOL and similar to immune or partial resistance to race 2. Only Aguamiel cultivar presented a SI reaction to FOL race 3. None of the cultivars presented a SI reaction to the three races. It can be concluded that failures on the control of the disease in this region can be attributed to the majority use of cultivars with incomplete resistance to the three FOL races. The seed packages also contain incomplete information about FOL resistance.

Keywords: *Solanum lycopersicum; Fusarium oxysporum* f. sp. *lycopersici*; race 3; immunity; partial resistance.

INTRODUCTION

Nova Friburgo city, a major tomato producer during summer, contributes to 13% of the production in the State of Rio de Janeiro (Emater-Rio, 2020). This production is concentrated in mountains areas of intensive management by family farmers. They have used hybrid cultivars of long-life type, reported as resistant to races 1 and 2 of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and, to a smaller extent, resistant to race 3. The use of resistant cultivars constitutes the most economical and easy to use form, besides not causing damage to the environment (Mcgovern, 2015). For this reason, it has been the main control strategy adopted by farmers in the studied region.

However, severe wilt losses and unviable areas for tomato growth are recurrent in this region. Among the wilt diseases, the Fusarium wilt, caused by FOL, is one of the most important tomato diseases worldwide (Dordevic *et al*., 2012; Mcgovern, 2015). Fusarium wilt can cause severe losses in protected crops and in the field (Dordevic *et al*., 2012).

The pathogen, which can be transmitted by seeds, causes damage to seedlings, and during the flowering and fruiting stages (Reis & Boiteux, 2007). The pathogen penetrates through the root system and colonizes the xylem vessels resulting in partial or complete disruption

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of water and nutrients, deposition of gums, thyllos, spores and mycelia, and visible discolouration of vascular tissue, leaf yellowing, wilt and death of plants (Mcgovern, 2015; Srinivas *et al*., 2019).

The three races of the pathogen are described by the ability to infect differential cultivars with different resistance loci, designated as immunity gene (*I*), introgressed from wild species (Reis *et al*., 2005). Among the identified R genes introgressed in *S. lycopersicum* are *I* and *I-1*, which confers resistance to race 1 and *I-2* which confers resistance to race 2, both from *S. pimpinellifolium*; *I-3* gene that confers resistance to race 3 and *I-7*, which confers resistance to races 1, 2 and 3, both from *S. pennellii* (Inami *et al*., 2012, Gonzalez-Cendales *et al*., 2016). In addition, the resistance gene governed by *I*, which confers immunity reaction to the respective races of FOL, is also reported as partial resistance resulting in delayed infection and colonization and less severe symptoms compared to a susceptible pattern. Partial resistance genotypes often referred to tolerant, are identified by inoculation tests with isolates of races 1 and 2 of FOL and classified as highly or moderately resistant (HR and MR) (Reis *et al*., 2004; Carrer-Filho *et al*., 2016).

The predominance of resistant cultivars to races 1 and 2 of FOL is probably due to the identification of these races for more than 70 years and also to the widespread distribution in Brazil and in the world (Inami et al., 2012; Gonzalez-Cendales et al., 2016; Gonçalves et al., 2020). As race 3 is a more recent finding, first reported in 1978 in Australia and only in 2005 in Brazil (Reis et al., 2005), the availability of resistant cultivars in Brazil is still limited. However, after the first report of race 3 in Venda Nova do Imigrante in Espírito Santo by Reis et al. (2005), several others reports have already been done in Brazil, as in Itaocara, RJ (Reis & Boiteux, 2007), Zona da Mata, MG (Gonçalves et al., 2013) and Jaguaquara, BA (Barboza et al., 2013), revealing the need to invest in resistant cultivars to this race as well. However, even using resistant cultivars to races 1 and 2 or to the three races, wilting symptoms are often reported, which leads to doubts about the nature and/or spectrum of this resistance to FOL races in this cultivars.

Therefore, considering the report of the widespread occurrence of Fusarium wilt in Nova Friburgo, RJ, even with the use of cultivars reported as resistant to the pathogen, this work was developed. The study aimed to evaluate the incidence and distribution of the disease in the region and to identify the resistance spectrum of the cultivars used to the FOL races, type and level of this resistance.

MATERIAL AND METHODS

Field harvest

Plant samples were collected in 40 tomato farms located in three association of producers in Nova Friburgo, RJ: Serra Nova, Serra Velha and Rio Grande (22º 17 ‘14’ S latitude, -42º 32 ‘01’’ W longitude and average annual temperature of 18.8 °C). During the surveys, the cultivars were identified, wilt occurrences and samples of plants with incipient and advanced symptoms of wilt and healthy or asymptomatic plants were harvested (table 1).

Fusarium identification and isolation

The plants were evaluated for the presence of vascular discolouration in the Laboratory of Epidemiology and Seed Pathology of UFRRJ (LabEPS). A standard procedure for fungal isolation was applied: aseptic removal of fragments from the xylem and superficial disinfestation (Baysal *et al*. 2009). Then, fragments of the vascular region of all plants were collected for isolation in PDA (potato-dextrose-agar) medium (Dinghra & Sinclair, 2000). The isolates were identified using the morphological characteristics and dimensions of the colonies and macroconidia, microconidia and chlamydospores with the aid of a stereoscopic and optical microscope (Booth 1977; Nelson *et al*. 1983). The isolates identified as F. oxysporum were inoculated in tomato seedlings (PAB cultivar) followed by reisolation in pure culture and preserved in soil and mineral oil for further studies (Dinghra & Sinclair, 2000).

Cultivars studied

The level of resistance to races 1, 2 and 3 of FOL were evaluated in 21 cultivars. The cultivars Lumi, Natália, Carina Ty, Ivety, Tyler and Débora Plus (Sakata, 2020), Siluety, Forty (Syngenta, 2020), Itaipava F_1 and Serato F_1 (Agristar, 2020), Alambra (Clause, 2020) and Carolina (Feltrin Sementes, 2017) - reported by the companies as resistant to races 1 and 2; Aguamiel (Vilmorin, 2017), TSV770Cromo (TecnoSeed, 2017) and BRS-Imigrante (Agrocinco, 2020) - reported as resistant to races 1, 2 and 3; San Marzano (ISLA, 2020) and Perinha Água Branca PAB (LabEPS) - with no information about the resistance to FOL races. Caeté (Blueseeds, 2020), Diana and Giuliana (Sakata, 2020), Juliete F_1 (Johnny’s, 2020), Paron (Syngenta, 2020) and Pizzadoro (Nunhems, 2020) cultivars were not evaluated to the resistance of FOL races due to unavailability of seeds in the producer region.

Table 1: List of tomato cultivars, company with respective information regarding the comercial group and resistance to the races 1, 2 and 3 of *Fusarium oxysporum* f. sp. *lycopersici* (FOL). FOL incidence was obtained from the plants diagnose *in loco* and using differential cultivars to the races of the pathogen

Genotype	Company	Group	FOL resistance according to the company's information	FOL incidence in the genotypes/farming	Total of genotypes with FOL
Aguamiel[1]	Vilmorin	Italiano	1, 2, 3	0	0
Alambra[1]	Clause	Salada	1, 2	F14(3), F27(1)	4
BRS Imigrante[2]	Agrocinco	Salada	1, 2, 3	-	-
Caeté[1]	Blueseeds	Saladete	1, 2	0	0
Carina Ty[2]	Sakata	Santa Cruz	1, 2	-	-
Carolina[2]	Feltrin	Cereja	1, 2	-	-
Débora Plus[1]	Sakata	Santa Cruz	1, 2	0	0
Diana[1]	Sakata	Salada	1, 2	0	0
Forty[2]	Syngenta	Salada	1, 2	-	-
Giuliana[1]	Sakata	Italiano	1	F10(1)	1
Itaipava F_1[1]	Agristar	Salada	1, 2	0	0
Ivety[2]	Sakata	Salada	1, 2	-	-
Juliete F_1[1]	Johnny's	Cereja	Not informed	F31(2)	2
Lumi[1]	Sakata	Salada	1, 2	0	0
Natália[1]	Sakata	Salada	1, 2	0	0
PAB[2]	LabEPS	Cereja	Not informed	-	-
Paron[1]	Syngenta	Salada	1, 2	0	0
Pizzadoro[1]	Nunhems	Italiano	1, 2	0	0
San Marzano[2]	Isla	Italiano	Not informed	0	-
Serato F_1[1]	Agristar	Salada	1, 2	F5(1), F7(2), F11(1), F13(3), F29 (2), F33(1)	10
Siluet[1]	Syngenta	Salada	1, 2	F6(1), F8(1), F9(2), F17(2)	6
TSV770 Cromo[1]	TecnoSeed	Salada	1, 2, 3		0
Tyler[2]	Sakata	Salada	1, 2		-
Ponderosa[3]	LabEPS	Salada	Susceptible		-
Caline IPA-7[3]	LabEPS	Salada	1		-
Floradade[3]	LabEPS	Salada	1, 2		-
LA-716[3]	LabEPS	*S. pennellii*	1, 2, 3		-
Total				14	23

[1]Cultivars used in Nova Friburgo. [2]Other comercial cultivars and genotypes from the LabEPS germoplasm bank; [3]Differential cultivars; [4]40 farms were evaluated in the study and 6 plants were harvested in each farm, three of them with wilted symptoms and three apparently healthy.

As a control, differential cultivars were used: Ponderosa (susceptible to races 1, 2 and 3), Caline IPA 7 (resistant to race 1), Floradade (Resistant to races 1 and 2) and *Solanum pennellii* LA-716 (resistant to races 1, 2 and 3). For the assays, four different FOL isolates were used: MMBF 01/96 (race 1, 5'-GTAACCCATATTGCGTGTTTCCCGGCCG-CCGCACGT-3') (MT846894 - GenBank accession number) and MMBF 02/96 (race 2, 5'-GTAACCCATATTG-CGTGTTTCCCGGCCGCCGCACGT-3') (MT846898 - GenBank accession number), from the Collection of Fungal Cultures Micoteca "Mário Barreto Figueiredo", Instituto Biológico, SP; FUS 2903 (race 3, 5'-GTAAC-CCATATTGCATGTTTCCCGGCCGCCGCACGT-3') (MT846888 - GenBank accession number) and FUS 1405 (race 3, 5'-GTAACCCATATTGCATGTTTCCCGGC-CGCCGCACGT-3') (MT846904 - GenBank accession number) from the UFRRJ LabEPS collection.

Experimental conditions and FOL inoculation

The experiments were performed in growth chamber (28/25 ºC day/night, relative humidity, 70%). A completely randomized experimental design was performed with 21 cultivars. Each genotype was represented by 10 plants, seven inoculated with the FOL races (1, 2 or 3) and three as control. The experiment was repetead at least three times to verify its reproducibility. The seedlings from each cultivar were germinated in the greenhouse and inoculated at 20 days after sowing.

The inoculation was done by cutting the tip of the seedling roots of the respective cultivars, 20 days after sowing. For the inoculation, conidial suspension containing $1x10^6$ microconidia. mL^{-1} of each respective isolate were used and water as a control. The roots were submerged in the conidial suspension for 5 minutes and then transplanted into tubes containing an autoclaved mixture of soil, sand, and substrate (1: 1: 1). After transplantation, 2.0 ml of the suspension was added to the respective tubes. Weekly, the plants were fertilized with 5.0 mL of Hoagland & Arnon solution (1950). For the control, distilled water was added, and the same procedures were adopted.

Analysis performed

At 18 days after inoculation, the plants were harvested and the fresh weight (g) of the shoot and the length (cm) of the stems were determined. Then, the stems were sectioned to measure the extension of vascular necrosis (EN) and grades (G) were attributed according to a scale proposed by Santos (1997), where: (1) plants without symptoms; (2) plants without symptoms of wilt or yellowing, but with vascular browning; (3) plants with vascular browning and wilt or foliar yellowing; (4) intensively wilted plants, associated with yellowing and foliar necrosis; (5) dead plants.

The relative fresh weigth (RFW), was estimated by the relation between fresh weigth data of inoculated plants (FWI) and non-inoculated plants - control (FWC) where, RFW = (FWI / FWC) * 100. Relative lenght (RL) was estimated by the relation between stems lenght data of the inoculated plants (SLI) and the controls (SLC), where RL = SLI / SLC * 100.

The percentage of vascular infection (PVI) was determined using the data from SL and the extent of necrosis (EN), where PVI = (EN / SL) * 100. The cultivars were classified using the grade average (G), according to model developed by Reis *et al*. (2004), where: 1.0 = similar to immune (SI); 1.1-2.0 = highly resistant (HR); 2.1-3.0 = moderately resistant (MR); 3.1-4.0 = susceptible (SU); 4.1-5.0 = highly susceptible (HS).

The incidence of plants with disease symptoms was determined by the relation between infected plants and total of inoculated plants, considering each race of the pathogen.

Statistical analysis

The Lilliefors test for normality of errors and Cochran test for homoscedasticity of variances was carried out on all variables studied (Neter *et al*., 1974). Subsequentely, the data was submitted to analysis of variance (ANOVA) by the F test and the means grouped by Scott-Knott test ($p < 0.05$), using the SISVAR program (Ferreira, 2000).

RESULTS AND DISCUSSION

The use of 15 different cultivars were reported to be used by the farmes in the producer's regions visited. The cultivars were: Aguamiel, Alambra, Caeté, Débora Plus, Diana, Giuliana, Itaipava F_1, Juliet F_1, Lumi, Natália, Paron, Pizzadoro, Serato F_1, Siluet and TSV770Cromo (Table 1). Some farmes planted more than one cultivar in the area. Others genotypes available in the LabEPS were also included in this table and in the subsequent analysis (BRS Imigrante, Carina T_y, Carolina, Forty, Ivety, PAB, San Marzano and Tyler) in order to confirm or identify it's FOL resistance.

The occurrence of Fusarium wilt caused by FOL was verified in 14 farmers from the 40 farmers analysed, approximately 35% of the area. These occurrences were recorded mainly in properties with Serato F_1. The farmers from F5, F7, F11, F13, F29 and F33 presented 10 plants in total infected with FOL, which corresponds to 42.9% from the farmers with FOL incidence.

Siluet presented FOL incidence in the farmers F6, F8, F9 and F17 and a total of 6 infected plants (28.6%), followed by Alambra with FOL incidence in two farmers F14 and F27 with 4 infected plants (14.3%). Giuliana and Juliet presented FOL incidence only in one farmer, F10 with one plant and F31 with two infected plants, respectively which represents a rate of 7.1%. For the other genotypes analysed, Fusarium wilt was not observed in theses farmers (Table 1).

Among the 17 cultivars evaluated, 14 displayed an incompatible reaction with FOL race 1, with SI reaction (I = 0, PVI = 0, G = 1), including all cultivars used in

Nova Friburgo and 5 cultivars from LabEPS bank (BRS Imigrante, Carina T_y, Forty, Ivety and Tyler) (Table 2). These results agree with the description available in the catalogues of the respective companies (Table 1). Only three cultivars displayed a compatible reaction, with SU reaction, Carolina (I = 85%, PVI = 80%, G = 3.6), and HS reaction, PAB (I = 100%, PVI = 93%, G = 4.1) and San Marzano (I = 100%, PVI = 100%, G = 4.9) (Table 2). Carolina cultivar is informed as resistant to races 1 and 2 of FOL by its catalogue, however it behaved as susceptible to race 1 in our work (Table 1).

The relationship between the three FOL races and tomato cultivars (*I-1*, *I-2* and *I-3*), is race-specific and follows the gene-to-gene theory with incompatible reaction (SI) and, therefore, independent of the isolate (Inami *et al*., 2012). However, the partial resistance may vary as a function of the FOL isolate aggressiveness (Carrer-Filho *et al*., 2016) and consistent results are obtained when more than one pathogen isolate is used (Souza *et al*., 2010; Akram *et al*., 2014).

Among the 17 cultivars tested, 15 are reported as resistant to race 2. However, only seven displayed an incompatible reaction with race 2, i.e, SI reaction (I = 0%, PVI = 0%, G = 1.0: BRS Imigrante, Forty, Ivety, Lumi, Natália,

Table 2: Percentage of infected plants (I%), percentage of vascular system infection (PVI %), severity grade (G) and classification regarding the resistance (R) from 17 tomato cultivars and 4 differential cultivars, inoculated with four *Fusarium oxysporum* f. sp. *lycopersici* (FOL) isolates from races 1, 2 and 3

Genotype	Race 1 (MMBF 01/96)				Race 2 (MMBF 02/96)				Race 3 (FUS 2903)				Race 3 (FUS 1405)			
	I (%)	PVI (%)*	G*	R	I (%)	PVI (%)*	G*	R	I (%)	PVI (%)*	N	R	I (%)	PVI (%)*	N*	R
Aguamiel[1]	0	0 c	1.0 d	SI	86	27 b	2.7 b	MR	0	0 c	1.0 c	SI	0	0 d	1.0	SI
Alambra[1]	0	0 c	1.0 d	SI	57	11 b	2.4 c	MR	100	84 a	4.1 a	HS	100	100 a	3.7	SU
BRS Imigrante[2]	0	0 c	1.0 d	SI	0	0 c	1.0 a	SI	100	48 b	3.4 b	SU	43	24 c	1.7	HR
Carina T_y[2]	0	0 c	1.0 d	SI	86	74 a	3.1 b	SU	100	90 a	4.3 a	HS	100	91 a	4.1	HS
Carolina[2]	85	80 b	3.6 c	SU	100	61 a	3.7 a	SU	100	100 a	4.4 a	HS	100	79 a	4.1	HS
Débora Plus[1]	0	0 c	1.0 d	SI	28	3c	1.4 a	HR	100	100 a	4.1 a	HS	100	93 a	3.5	SU
Forty[2]	0	0 c	1.0 d	SI	0	0 c	1.0 a	SI	100	100 a	4.6 a	HS	100	92 a	3.4	SU
Itaipava F_1[1]	0	0 c	1.0 d	SI	28	19 b	1.3 a	HR	100	91 a	3.9 a	SU	100	93 a	3.8	SU
Ivety[2]	0	0 c	1.0 d	SI	0	0 c	1.0 a	SI	100	88 a	4.0 a	SU	100	92 a	3.9	SU
Lumi[1]	0	0 c	1.0 d	SI	0	0 c	1.0 a	SI	100	89 a	4.1 a	HS	100	73 a	3.1	SU
Natália[1]	0	0 c	1.0 d	SI	0	0 c	1.0 a	SI	86	74 b	3.4 b	SU	100	89 a	4.0	SU
PAB[2]	100	93 a	4.1 b	HS	43	35 b	2.3 c	MR	100	81 a	4.4 a	HS	100	81 a	4.0	SU
San Marzano[2]	100	100 a	4.9 a	HS	100	94 a	4.1 a	HS	100	97 a	4.6 a	HS	100	100 a	4.4	HS
Serato F_1[1]	0	0 c	1.0 d	SI	0	0 c	1.0 a	SI	100	100 a	4.4 a	HS	100	93 a	3.4	SU
Siluet[1]	0	0 c	1.0 d	SI	57	18 b	2.1 c	MR	100	84 a	4.4 a	HS	100	83 a	4.4	HS
TSV770-Cromo[1]	0	0 c	1.0 d	SI	0	0 c	1.0 a	SI	86	62 b	3.3 b	SU	71	41 b	1.7	HR
Tyler[2]	0	0 c	1.0 d	SI	86	17 b	3.0 b	MR	100	96 a	4.6 a	HS	100	86 a	4.0	SU
Ponderosa[3]	100	100 a	4.8 a	HS	100	88 a	4.4 a	HS	100	100 a	4.9 a	HS	100	100 a	4.6	HS
Caline IPA-7[3]	0	0 c	1.0 d	SI	86	75 a	3.6 a	SU	100	100 a	4.0 a	SU	100	100 a	4.7	HS
Floradade[3]	0	0 c	1.0 d	SI	0	0 c	1.0 a	SI	100	66 b	3.0 b	SU	100	72 a	3.7	SU
LA-716[3]	0	0 c	1.0 d	SI	0	0 c	1.0 a	SI	0	0 c	1.0 c	SI	0	0 d	1.0	SI
CV%		29.6	4.7			54.8	12.7			17.8	6.8			18.3	7.65	

[1]Cultivars used in Nova Friburgo, [2]Other comercial cultivars and genotypes from the LabEPS germoplasm bank and [3]Differential cultivars; Mean followed by the same lower-case letter in the column do not differ statistically by the Scott-Knott test at 5% of probability. I = Incidence of Fusarium wilt, expressed in percentage; PVI = Percentage of vascular infection; G = Severity grade and R = resistance determined, where SI= 1.0; HR=1.1-2.0; MR= 2.1-3.0; SU= 3.1-4.0; HS= 4.1-5.0.

TSV770Cromo and Serato F_1 (Table 2). Two other cultivars were identified as HR, Débora Plus (I = 28%, PVI = 3%, G = 1.4) and Itaipava F_1 (I = 28%, PVI = 19% and G = 1.3). Other four were identified as MR: Aguamiel (I = 86%, PVI = 27%, G = 2.7), Alambra (I = 57%, PVI = 11%, G = 2.4), Siluet (I = 57%, PVI = 18%, G = 2.1) and Tyler (I = 86%, PVI = 17%, G = 3.0). The PAB variety, previously uncharacterized, showed MR reaction (I = 43, PVI = 35%, G = 2.3). Two of these cultivars, Carina T_y (I = 86%, PVI = 74%, G = 3.1) and Carolina (I = 100%, PVI = 61%, G = 3.7), although reported as resistant to races 1 and 2, also behaved as susceptible (SU) to race 2. San Marzano, with no information on the reaction to FOL race 2, behaved as highly susceptible HS (I = 100, PVI = 94%, G = 4.1) (Table 2).

Based on these results, it can be inferred that from the 15 cultivars marketed as resistant to race 2 of FOL, only seven (BRS Imigrante, Forty, Ivety, Lumi, Natália, Serato F_1 and TSV770Cromo) have complete resistance characterized by similar to immune (SI) (Table 2). Another six are partially resistant, two of them highly resistant (HR) (Débora Plus and Itaipava F_1) and four moderately resistant (Aguamiel, Alambra, Siluet and Tyler). Two, although reported as resistant, are susceptible (SU) to race 2 of FOL (Carina T_y and Carolina) (Table 2). San Marzano and PAB cultivars, with no information available regarding FOL resistance, were characterized. San Marzano was considered highly susceptible to races 1 and 2, while PAB was partially resistant to FOL race 2 (Table 2).

The cultivars Alambra, Serato F_1 and Siluet are reported by the companies as resistant to FOL races 1 and 2. However, presented plants infected with FOL. This result led us to analyse two possibilities, first - these cultivars are not resistant to race 1 or 2 of FOL and second – this region presents the dissemination of FOL race 3 that can infect plants with only resistance to race 1 and 2 of FOL.

From the 15 cultivars studied and reported as resistant to race 2 (table 2), only seven displayed an incompatible reaction to race 2, including Serato F_1 cultivar. These same cultivars showed SI reaction to race 1, which confirms the simultaneous introgression of the genes *I-1* and *I-2* (Carrer-Filho *et al*., 2016). Alambra and Siluet cultivars presented incompatible reaction to race 1 (SI) and moderately resistance to race 2. Based in these results, Alambra and Siluet cultivars could be infected with race 2 or 3 of FOL, while Serato F_1 could be infected only by race 3 of FOL. This result indicates that the second possibility afore mentioned is the most presumable and the area could be contaminated with FOL race 3. Oliveira *et al*. (2021) have characterized the isolates obtained from the producer's area in Nova Friburgo region and confirmed by molecular tests the existence of FOL race 3.

Finally, three of the 17 cultivars tested are reported as resistant to race 3 (Aguamiel, TSV770Cromo and BRS Imigrante). However, only one, Aguamiel, presented an incompatible reaction with the two isolates of FOL race 3 (FUS 2903 and FUS 1405), similar to LA-716 with SI reaction (G = 1, I = 0%, PVI = 0%). The two other cultivars related as resistant to race 3, TSV770Cromo and BRS Imigrante, showed a compatible response to the two FOL isolates. These reactions were identified as susceptibility, SU, to the FUS 2903 isolate in TSV770Cromo (I = 86%, PVI = 62, G = 3.3) and BRS Imigrante (I = 100%, PVI = 48%, G = 3.4) and as a high resistance, HR, to the isolated FUS 1405 in TSV770Cromo (I = 71%, PVI = 41, G = 1.7) and BRS Imigrante (I = 43, PVI = 24, G = 1.7). All other cultivars exhibited compatible response and susceptibility (SU) or high susceptibility (HS) responses to the two isolates of race 3 (I = 100%, PVI > 73, G > 3.0) (Table 2).

This result is in accordance with the survey of Mcgovern (2015) and the finding that most tomato cultivars have resistance only to races 1 and 2 of FOL. Several improved tomato cultivars present resistance to race 1 and 2 of FOL and a small number possess resistance to the three races (Reis et al., 2005; Mcgovern, 2015).

None of the 17 cultivars tested showed complete resistance, SI reaction, to the three FOL races. The only cultivar resistant to race 3, showed SI reaction to race 1 and MR only to race 2 (Table 2). From the information available in the seed packages, only in 46% of the cultivars, the resistance to race 2 was confirmed as being of immunity and, in another 46% as being of a partial nature, HR, MR or susceptible reaction (Table 2).

It is importante to consider that the resistance of cultivars to FOL is conditioned to the presence of the genes *I* and *I-1* (race 1), *I-2* (race 2), *I-3* (race 3) and *I-7*, that promotes resistance to the three races of FOL (Gonzalez-Cendales *et al*., 2016; Biju *et al*., 2017). These genes can be introgressed simultaneously or independently. Thus, resistance will depend on the genetic configuration of tomato cultivars.

These results also reveal an emerging problem in one of the main tomato-producing regions of Brazil, the possibility of the presence of FOL race 3 and reduced supply of resistant cultivars. Another point to consider, is the imprecision in the information contained in the company catalogues and the non-citation of the genes related to the respective reported resistances.

The plant age, methodology and environmental conditions can also affect the results of genotype characterization for partial resistance (Boix-Ruíz *et al*., 2015). The methodology adopted is the same used by different authors (Souza *et al*., 2010, Carrer-Filho *et al*., 2016; Gonçalves *et al*., 2020), as well as the initial screening and identification of SI reactive materials. However, as it is a drastic method it may hinder the identification of genotypes bearing partial resistance. The stability of this resistance must be verified in field studies and evaluated until the adult plant (Cantú *et al*., 2014).

In general, the inoculations using four isolates resulted in a significant reduction of the fresh weight and plant growth, even in incompatible interactions, where loss from 50% to 80% were registered compared to the non-inoculated plants. Higher losses, however, were observed in the plants of the cultivars with HS reaction - Ponderosa (60 to 90%). Differences were also observed between isolates, with higher losses caused by the FUS 2903 isolate of race 3 and lower by the isolate MMBF 02/96 of race 2 (Table 3).

Table 3: Relative fresh weight of shoots (RFW) and relative lengh of stems (RL) from tomato plants inoculated with four *Fusarium oxysporum* f. sp. *lycopersici* isolates, races 1, 2 and 3, compared with non-inoculated plants

Genotype	Race 1 (MMBF 01/96)		Race 2 (MMBF 02/96)		Race 3 (FUS 2903)		Race 3 (FUS 1405)	
	RFW %	RL%	RFW %	RL%	RFW %	RL%	RFW %	RL%
Aguamiel[1]	81 b	99 a	65 b	84 b	88 a	92 a	88 b	122 b
Alambra[1]	104 a	91 a	83 a	96 a	36 c	43 c	44 c	38 c
BRS Imigrante[2]	95 a	89 b	92 a	93 a	59 c	63 b	104 b	129 b
Carina T_y[2]	64 b	71 b	37 c	51 c	22 d	27 c	41 c	66 b
Carolina[2]	36 c	52 c	54 c	71 b	13 d	25 c	32 c	45 c
Débora Plus[1]	78 b	88 a	85 a	102 a	19 d	36 c	54 c	71 b
Forty[2]	60 b	73 b	75 b	96 a	41 c	42 c	49 c	49 c
Itaipava F_1[1]	97 a	107 a	70 b	91 a	29 d	46 b	33 c	37 c
Ivety[2]	107 a	113 a	101 a	99 a	41 c	63 b	49 c	93 b
Lumi[1]	85 b	90 a	89 a	80 b	42 c	50 b	65 c	72 b
Natália[1]	70 b	92 a	97 a	97 a	40 c	59 b	38 c	58 c
PAB[2]	29 c	40 c	75 b	76 b	13 d	16 c	28 d	29 d
San Marzano[2]	2 d	7 d	39 c	45 c	12 d	19 c	26 d	46 c
Serato F_1[1]	82 b	89 a	85 a	90 a	24 d	37 c	51 c	60 c
Siluet[1]	92 a	100 a	73 b	93 a	25 d	34 c	29 d	27 d
TSV770-Cromo[1]	49 c	61 c	81 a	82 b	42 c	51 b	90 b	80 a
Tyler[2]	106 a	108 a	65 b	77 b	20 d	27 c	42 c	48 c
Ponderosa[3]	5 d	9 d	24 c	33 c	4 d	8 c	8 d	17 d
Caline IPA-7[3]	34 c	50 c	39 c	52 c	31 d	49 b	9 d	10 d
Floradade[3]	104 a	95 a	91 a	84 b	47 c	41 c	56 c	47 c
LA-716[3]	89 a	82 b	71 b	83 b	60 b	67 b	159 a	122 a
CV%	28.4	24.5	24.5	19.6	49.9	45.4	39.3	37.7

RFW = (FWI/FWC)*100; RL = SLI/SLC*100; FWI = fresh weight of inoculated plants; FWC = fresh weight of non-inoculated plants; SLI = Stem lengh of inoculated plants and SLC = Stem lengh of non-inoculated plants. [1]Cultivars used in Nova Friburgo, [2]Other comercial cultivars and genotypes from the LabEPS germoplasm bank and [3]Differential cultivars.

Reduction in the development of susceptible and resistant FOL tomatoes is reported by Huang & Lindhout (1997) and Safiuddin *et al*. (2012). This reduction may be related to the plant defense response to the infection process.

Delay in the development of plants, either by the reduction in the accumulation of fresh weight or growth, is common in plants with Fusarium wilt, resulted from the restriction of xylem vessels due to the growth of hyphae and spore production within them as well (Gonzalez-Cendales *et al*., 2016). The reduction on the growth and biomass accumulation is more severe in the earlier infection and in resistant plants. The reduced growth may occur due to dysregulation of the primary metabolism with the defense response to infection (Rojas *et al*., 2014; Srinivas *et al*., 2019), wich justifies the results obtained.

The reaction observed from the commercial cultivars tested to the FOL isolates reveals the need of studies focusing on finding and produce resistant materials. Tokeshi *et al.* (1966) reported since this date the need of research that mainly target material of basic origin in breeding programs. Many conventionally improved tomato cultivars have resistance to races 1 and 2 of FOL and few have combined resistance to the three races (Mcgovern, 2015), as observed in our work.

CONCLUSION

Failures on the control of Fusarium wilt in Nova Friburgo, RJ, is due to the majority use of cultivars with incomplete resistance to the three FOL races.

Among all cultivars evaluated, only one - Aguamiel – displayed a similar to immune (SI) response to FOL race 3; two others, TSV770Cromo and BRS Imigrante, had a SU or HR reaction, depending on the aggressiveness of the FOL isolate; all others are susceptible or highly susceptible to race 3.

The information contained on the seed packages about FOL races resistance is incomplete and often does not necessarily correspond to the SI reaction.

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