horticultura brasileira

Research

ALBUQUERQUE, GR; LUCENA, LP; ASSUNÇÃO, EF; MESQUITA, JCP; SILVA, AMF; SOUZA, EB; NICOLI, A; GAMA, MAS. 2021. Evaluation of tomato rootstocks to *Ralstonia solanacearum* and *R. pseudosolanacearum* in Mata mesoregion, PE. *Horticultura Brasileira* 39: 072-078. DOI: http://dx.doi.org/10.1590/s0102-0536-20210111

Evaluation of tomato rootstocks to *Ralstonia solanacearum* and *R. pseudosolanacearum* in Mata mesoregion, PE

Géssyka R Albuquerque [1]; Lucas P Lucena [1]; Emanuel F Assunção [1]; Júlio Carlos P Mesquita [2]; Adriano Márcio F Silva [1]; Elineide B Souza [3]; Alessandro Nicoli [4]; Marco Aurélio S Gama [1]

[1]Universidade Federal Rural de Pernambuco, Departamento de Agronomia (UFRPE), Recife-PE, Brasil; gessyka.r@hotmail.com; lucaspontes94@hotmail.com; as_emanuel@hotmail.com; adrianomfsilva@yahoo.com.br; mas.gama@yahoo.com.br; [2]Instituto Agronômico de Pernambuco (IPA), Recife-PE, Brasil; julio.mesquita@ipa.br; [3]Universidade Federal Rural de Pernambuco, Departamento de Biologia (UFRPE), Recife-PE, Brasil; elineidebs@yahoo.com.br; [4]Universidade Federal dos Vales do Jequitinhonha e Mucuri, Instituto de Ciências Agrárias (UFVJM), Unaí-MG, Brasil; alessandro.nicoli@ufvjm.edu.br

ABSTRACT

Bacterial wilt limits tomato production and resistant rootstocks could be important for the integrated management of the disease. Since there is an interaction between local bacterial strains and tomato genotype, this study aimed to evaluate 14 tomato rootstocks to bacterial wilt in the Mata mesoregion of Pernambuco state, Brazil. The rootstocks reaction to two sequevars of *Ralstonia solanacearum* and two of *R. pseudosolanacearum* was evaluated in four experiments carried out in the greenhouse using the completely randomized experimental design, with four replications composed of four plants each. Seven genotypes were selected to evaluate the reaction to bacterial wilt as rootstocks grafting in tomato plants 'Tomini F1' in a production area with disease history in the Chã Grande municipality, using randomized block design with four plants per treatment in each block. In the field experiment, disease symptoms were not observed in the grafted plants in 'Guardião', 'Woodstock', and 'Yoshimatsu'. Regarding all experiments, 'Guardião' and 'Muralha' showed the best resistance levels and could be used in the integrated management of bacterial wilt and studied in plant breeding programs.

Keywords: *Solanum lycopersicum*, grafting, genetic resistance, resistance.

RESUMO

Avaliação de porta-enxertos de tomateiro a *Ralstonia solanacearum* e *R. pseudosolanacearum* na mesorregião da Mata de Pernambuco

A murcha bacteriana limita a produção do tomateiro sob condições de alta temperatura e umidade, e porta-enxertos resistentes são importantes para o manejo integrado da doença. Visto que existe interação entre isolados locais e genótipos de tomateiros, objetivou-se avaliar 14 porta-enxertos de tomateiro à murcha bacteriana na mesorregião da Mata de Pernambuco, Brasil. A reação dos porta-enxertos a dois sequevares de *Ralstonia solanacearum* e dois de *R. pseudosolanacearum* foi avaliada em quatro experimentos realizados em casa de vegetação em delineamento inteiramente casualizado, com quatro repetições, com quatro plantas cada. Sete genótipos foram selecionados para avaliar a reação à doença como porta-enxertos de tomateiro 'Tomini F1' em uma área de produção com histórico da doença em Chã Grande, Pernambuco, Brasil, utilizando delineamento em blocos casualizados, com quatro plantas por tratamento por bloco. Não foram observados sintomas da doença nas plantas enxertadas em 'Guardião', 'Woodstock' e 'Yoshimatsu'. Considerando todos os experimentos, 'Guardião' e 'Muralha' apresentaram os melhores níveis de resistência, podendo ser utilizados no manejo integrado da murcha bacteriana e estudados em programas de melhoramento.

Palavras-chave: *Solanum lycopersicum*, enxertia, resistência genética, resistência.

Received on June 2, 2020; accepted on November 11, 2020

The tomato crop stands out among the most important ones in the world agricultural scenario and Brazil occupies the 9th position in this ranking, with a production of about 4.1 million tons in 2018 (FAO, 2020). In 2019, the tomato harvest in the Northeast region of Brazil was approximately 518 thousand tons. The state of Pernambuco reached a production of approximately 47 thousand tons, being the third largest producer in the region (IBGE, 2020). However, several abiotic and biotic factors can limit tomato production, such as the occurrence of diseases and pests. In this context, although there were no data about losses in the Mata mesoregion of the Pernambuco state, the tomato may have its production limited due to the occurrence of bacterial wilt, which has been responsible for causing great losses in this crop and other cultivated solanaceous, being one of the main problems in tropical regions worldwide (Grover *et al.*, 2006). In addition, due to the difficulties faced with the phytosanitary problems of the tomato crop, cultivation areas have been abandoned by producers (Lopes, 2009). This disease can be caused by *Ralstonia solanacearum*, *R. pseudosolanacearum* and *R. syzygii* subsp. *indonesiensis*, although the latter is not present in

Brazil (Safni *et al*., 2014; Prior *et al*., 2016; Santiago *et al*., 2017; Lopes & Rossato, 2018) and several sequevars of the two first bacteria have been found causing bacterial wilt in Solanaceae in the Mata mesoregion of Pernambuco state, Brazil, turning the disease control difficult (Garcia *et al*., 2013).

The use of resistant or tolerant cultivars is one of the measures to control bacterial wilt of the Solanaceae, which is considered of extreme importance within the integrated management of the disease (Lopes *et al*., 2015). However, marketable resistant tomato cultivars are not available and resistant rootstocks have been used to suppress infection of susceptible plants (Nakaho *et al*., 2004). In turn, resistant rootstocks may significantly reduce the incidence and severity of bacterial wilt in tomato plantations and the genotypes 'Hawaii 7998', 'Cheong Gang', 'BHN 1054', 'BHN 998', 'RST-04-106-T' (McAvoy *et al*., 2012), 'Hawaii 7996' (Lopes *et al*., 2015; Caldwell *et al*., 2017), 'Guardião', 'Muralha' (Lopes *et al*., 2015), 'Green-guard' (Uehara & Nakaho, 2018), and 'Yoshimatsu' (Costa *et al*., 2018, 2019) have been reported as resistant or tolerant. On the other hand, different studies have shown that some hybrids may show a susceptibility when infected by different strains of *Ralstonia* spp., evidencing the importance of selecting new sources of resistance as well as the need to evaluate hybrids due to the different performance of rootstocks according to the strain and the environmental conditions (Rivard *et al*., 2012; Lopes *et al*., 2015; Kim *et al*., 2016; Lopes & Mendonça, 2016).

The resistance of the available rootstocks is not considered an immune response because it is only able to retard the pathogen development in xylem vessels (Grimault *et al*.,1994; Lopes *et al*., 2015; Caldwell *et al*., 2017), as for instance the genotype Hawaii 7996, which difficult the bacterial colonization in the vascular cylinder (Caldwell *et al*., 2017). Thus, this study aimed to assess the specificity reaction of 14 tomato rootstocks to bacterial wilt, caused by different sequevars of *R. solanacearum* and *R. pseudosolanacearum*, representative of the local variability, in the environmental conditions of the Mata mesoregion of Pernambuco state and to provide to the tomato producers a background about the rootstocks that may be used in this region.

MATERIAL AND METHODS

Ralstonia spp. strains and pathogenicity test

The strains used in this work were obtained from the Rosa Mariano Culture Collection of the Laboratory of Phytobacteriology (LAFIBAC) of Universidade Federal Rural de Pernambuco (UFRPE). Two *R. solanacearum* (RS) and two *R. pseudosolanacearum* RP strains were used, of different sequevars (Table 1). These strains were obtained from tomato plants at production regions in previous studies (Albuquerque *et al*., 2021).

The four strains of *Ralstonia* were grown in triphenyl tetrazolium chloride medium incubated at 30°C for 48 h, for selection of virulent colonies (Kelman, 1954). The preparation of bacterial suspensions was carried out in sterile distilled water (SDW), adjusting the concentration to 10^8 CFU mL^{-1} with the aid of a photocolorimeter (Analyser®).

The pathogenicity of the strains was evaluated in tomato seedlings cultivar IPA 6, grown in styrofoam trays with 200 cells. The seedlings were transplanted individually after 15 days into 500 mL plastic pots containing soil and commercial substrate (Basaplant®), in proportion to 3:1. After 30 days of sowing, the plants were inoculated with the deposition of 15 mL of the bacterial suspension (1,5 x 10^8 CFU mL^{-1}) on the substrate, where semicircle root injuries were performed. For comparative purposes, plants treated only with SDW were used as absolute control.

The plants were evaluated at 25 days after inoculation to determine the disease severity, when the wilt symptoms stabilized in the plants, according to Lopes *et al.* (2015). The evaluation was carried out with the aid of the scale descriptive of grades of Nielsen & Haynes (1960), ranging from 0 to 5, in which grade 0 was attributed to plants without symptoms, 1 to plants with a wilted leaf, 2 to plants with 1/3 of wilted leaves, 3 to plants with 2/3 of wilted leaves, 4 to wilted plants, and 5 to dead plants. The values obtained were transformed into disease index (DI), on what DI = [Σ (disease grade x grade frequency) / (total number of plants x maximum disease grade)] x 100 (McKinney, 1923). The experimental design used was completely randomized, with four replications composed of four plants each. The obtained data were checked according to ANOVA assumptions and the means compared by the LSD test ($P < 0.05$) with the aid of the program AgroEstat v.1.1.0.712 (Barbosa & Maldonado Júnior, 2015).

Tomato rootstocks reaction to bacterial wilt in greenhouse

The reaction of the 14 rootstocks was evaluated in relation to bacterial wilt (Table 2). For comparative purposes, the genotypes Hawaii 7996 and L390 were used as universal standards of resistance and susceptibility, respectively (Wang *et al*., 1998). The reaction of all 14 tomato genotypes was evaluated individually for each *Ralstonia* strain (Table 1), in four different experiments carried out from November to December 2017 (30ºC±2; RH 57%) and repeated from March to April 2018 (34ºC±2; RH 65%). The steps of planting, inoculation and evaluation were realized according to the previously described. These experiments were set up in a completely randomized design, using four replications composed of four plants each.

The incidence (INC) was calculated by the percentage of plants with disease symptoms in relation to the total number of plants, and the wilt severity, evaluated as previously described. Asymptomatic plants were analysed for latent infection caused by *R. pseudosolanacearum* and *R. solanacearum*, following the plate methodology proposed by Lebeau *et al.* (2011). The plates were incubated at 30ºC for 96 h, then the absence or presence of virulent typical colonies of *Ralstonia* spp. were observed (Kelman, 1954). The data obtained from the isolation of asymptomatic plants were used to calculate the colonization index (CI), on what CI = percentage of

wilted plants + (percentage of plants without symptoms x percentage of plants without symptoms but with latent infection) (Grimault *et al.*, 1994; Prior *et al.*, 1996).

Considering that no significant (P<0.05) differences were observed regarding variance of the two experiments, the data were evaluated as replicates in time. The assumptions of the analysis of variance (ANOVA) were verified by the Shapiro-Wilk and Levene's tests using the software Statistix 9 (v. 9.0, Tallahassee, Florida, USA). The means of the variables were analysed by the Scott-Knott test (P<0.05), with the aid of the program AgroEstat v.1.1.0.712 (Barbosa & Maldonado Júnior, 2015).

Tomato rootstocks reaction to bacterial wilt in an area with history of disease occurrence

Based on the results with artificial inoculations in the greenhouse, the rootstocks 'Guardião', 'Woodstock', 'Yoshimatsu', 'Tropithai', 'TD1', and 'Green Rise' were selected and grafted with the hybrid genotype cv. Tomini F1 (Feltrin®) and tested in a tomato commercial production area naturally infested with RS and RP (Garcia *et al*., 2013, 2014), in Chã Grande city, Pernambuco, Brazil, where the inoculum dispersion has been spread by irrigation water. For comparative purposes, we used the genotypes Hawaii 7996 and L390 as a standard of resistance and susceptibility, respectively. Also, plants of non-grafted tomato cv. Tomini F1 were included in the experiment.

Planting was performed using 200 cells trays, containing coconut fiber substrate. The grafting in the seedlings was realized 21 days after sowing when the seedlings were cut in bevel and joined with the aid of a clamp. After the grafting, the seedlings were incubated under high relative humidity for 15 days, being transplanted soon after to 20 L vases containing coconut fiber. The plants were maintained in an experimental field under protected cultivation and conducted on two stems, being drip-fertigated according to crop necessity, and sprayed with abamectin to control *Liriomyza* spp. and mites and thiamethoxam to control *Bemisia* spp. The disease evaluation was performed weekly until the fruit ripeness, at 60 days after planting. In addition to the symptoms, the presence of bacterial exudate was evaluated through dispersion in water from symptomatic plants at random.

The assessments and calculations of the disease index and incidence were performed as previously described. Additionally, the area under the disease progress curve (AUDPC) was calculated for each treatment according to Shaner & Finney (1977) based on seven assessments carried out once a week. The experimental design was a randomized block, with four blocks, each composed of four plants per treatment. The means of the variables were checked according to ANOVA assumptions and analysed by the LSD test (P<0.05) using Statistix v.9.0 (Tallahassee, FL). When necessary, transformations were made to meet the assumptions of the ANOVA.

RESULTS AND DISCUSSION

Pathogenicity test of *Ralstonia* spp. strains

The strains of the two species used in the greenhouse experiments were pathogenic and showed a high disease index (DI) (Table 1). A significant difference was observed only between strains CRMRS91 and CRMRS183, which presented DI of 100 and 90%, respectively. The results showed that the strains remained pathogenic during preservation and with aggressiveness like that observed by Albuquerque *et al.* (2021).

Tomato rootstocks reaction to bacterial wilt in the greenhouse

Regarding *R. solanacearum* and the strain CRMRS91, for the variable DI, the least infected rootstocks were 'Woodstock' (47.5%), 'Guardião' (48.12%), 'Muralha' (58.12%), 'Green Power' (61.87%), and 'BSPE0041' (66.87%), that did not differ from 'Hawaii 7996' (36.87%), considered as a universal standard of resistance (Table 3). In turn, for the variables INC and CI, only the 'Guardião' (68.75 and 69.37%) did not differ from 'Hawaii 7996' (65.62 and 66.62%). In the experiments carried out with the strain CRMRS183, for DI, INC, and CI, only 'Guardião' (39.37, 56.25, and 57.15%) and 'Muralha' (51.87, 65.62, and 65.99%) did not differ from 'Hawaii 7996' (31.87, 43.75, and 45.00%) and were considered resistant.

Regarding *R. pseudosolanacearum* and the strain CRMRS126, in the variable DI, the rootstocks 'Guardião' (20.62%), 'BSPE0039' (46.87%), 'Woodstock' (54.37%) and 'Green Barrier' (58.75%) did not differ from 'Hawaii 7996' (37.5%) (Table 4). However, considering the INC and CI, 'Guardião' (25.00 and 25.62%) displayed the least disease when compared to the other genotypes, significantly surpassing 'Hawaii 7996'. In turn, when the strain CRMRS116 was inoculated, for the variable DI, the 'Guardião' (0.00%), 'Woodstock' (0.00%), and 'Muralha' (0.00%) showed a higher level of resistance in relation to 'Hawaii 7996' (10.62%), but did not differ from each other. For the variables INC and CI, the genotypes 'Guardião' (0.00%; 0.56%), 'Woodstock' (0.00%; 0.31%), and 'Muralha' (0.00%; 0.53%)

Table 1. Description of the strains of *Ralstonia solanacearum* (RS) and *Ralstonia pseudosolanacearum* (RP) used in this study for pathogenicity. Recife, UFRPE, 2017.

Strain	Species	City	Sequevar[1]	DI (%)[2]
CRMRs91	*Ralstonia solanacearum*	Camocim de São Félix	IIA-58	100.00 a
CRMRs116	*R. pseudosolanacearum*	Gravatá	I-18	98.70 ab
CRMRs126	*R. pseudosolanacearum*	Belém de São Francisco	I-17	98.70 ab
CRMRs183	*R. solanacearum*	Petrolina	IIA-50	90.00 b
CV (%)	-	-	-	10.0

The values represent means of four replications. Means with same letter are not significantly different by LSD test (P<0.05). [1]Source: Albuquerque *et al*. (2021); [2]DI = Disease index.

displayed the least disease, differing from 'Hawaii 7996' (25.00%; 25.01%) and the other genotypes. However, although such hybrids have shown symptoms, once some resistance level is observed, it is an indication that the rootstock can be promising, even in areas with soils infested by *Ralstonia* spp. In this context, it is essential to carry out complementary field tests (McAvoy *et al.*, 2012; Rivard *et al.*, 2012).

The INC was considered high in the four experiments and when the rootstocks were tested with the strains of *R. solanacearum*, a higher mean DI value was observed. The strains of the two species were also able to colonize the xylem of all evaluated genotypes, even in asymptomatic plants, as demonstrated by the CI. Strains of *Ralstonia* spp.

Table 2. Resistance of the tomato rootstocks to diseases. Recife, UFRPE, 2020.

Rootstock	Company	Resistance[1]
Guardião	Takii Seed	Rs, Vd, Fol (race 1, 2), For, ToMV, Ma, Mi, Mj
Muralha	Takii Seed	Rs, Vd, Fol (race 1, 2), For, ToMV, Ma, Mi, Mj
TD1	Takii Seed	Rs, Vd, Pl, Fol (race 1, 2, 3), For, ToMV, Ma, Mi, Mj
Green power	Takii Seed	Rs, Vd, Pl, Fol (race 1, 2, 3), For, ToMV, Ma, Mi, Mj
Green barrier	Takii Seed	Rs, Vd, Pl, Fol (race 1, 2, 3), For, ToMV, Ma, Mi, Mj
Green rise	Takii Seed	Rs, Vd, Pl, Fol (race 1, 2, 3), For, ToMV, Ma, Mi, Mj
Yoshimatsu	Inpa	Rs
Woodstock	Sakata Seed	Rs, Vd (race 1), Fol (race 1, 2), For, ToMV, Mi (race 1, 2, 3, 4), Mj
Defensor F1	Topseed	Rs,Vd, Fol (race 2), For, ToMV, Mi
RZ01	Rijk Zwaan	NI
RZ02	Rijk Zwaan	NI
BSPE0039	Blue seeds	NI
BSPE0041	Blue seeds	*Ralstonia*, *Verticillium* (race 1), Fol (race 1, 2, 3), For, *Phytophthora* root rot, *Meloidogyne*
Tropithai F1	East-West Seed	NI

[1]Informations obtained on the company's websites. Rs= *Ralstonia solanacearum*; Vd= *Verticillium dahliae*; Pl= *Pyrenochaeta lycopersici*; Fol= *Fusarium oxysporum* f. sp. *lycopersici*; For= *Fusarium oxysporum* f. sp. *radicis-lycopersici*; ToMV= *Tomato mosaic virus*; Ma= *Meloidogyne arenaria*; Mj= *Meloidogyne javanica*; Mi= *Meloidogyne incognita*; NI= No information obtained on the company website.

Table 3. Reaction of tomato rootstocks to bacterial wilt caused by two sequevars of *Ralstonia solanacearum*. Recife, UFRPE, 2017/2018.

Genotype	CRMRS91 (IIA-58)			CRMRS183 (IIA-50)		
	DI (%)[1]	INC (%)	CI (%)	DI (%)	INC (%)	CI (%)
BSPE0039	75.62 b[2]	87.50 a	87.50 a	81.87 b	84.37 a	84.37 a
BSPE0041	66.87 c	87.50 a	87.50 a	75.00 b	84.37 a	84.37 a
Defensor	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
Green Barrier	71.87 b	90.62 a	90.62 a	74.37 b	90.62 a	90.62 a
Green Power	61.87 c	84.37 a	84.37 a	65.62 b	93.75 a	93.75 a
Green Rise	74.37 b	90.62 a	90.62 a	83.12 b	93.75 a	93.75 a
Guardião	48.12 c	68.75 b	69.37 b	39.37 c	56.25 b	57.15 b
Hawaii 7996	36.87 c	65.62 b	66.62 b	31.87 c	43.75 b	45.00 b
L 390	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
Muralha	58.12 c	84.37 a	84.87 a	51.87 c	65.62 b	65.99 b
RZ01	73.75 b	93.75 a	93.75 a	81.25 b	96.87 a	96.87 a
RZ02	79.37 b	87.50 a	87.50 a	88.75 a	93.75 a	93.75 a
TD1	83.12 b	90.62 a	90.62 a	80.62 b	93.75 a	93.75 a
Tropithai	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
Woodstock	47.50 c	84.37 a	84.37 a	66.87 b	84.37 a	84.5 a
Yoshimatsu	91.25 a	93.75 a	93.75 a	86.25 b	96.87 a	96.87 a
CV (%)	30.00	21.00	20.00	29.00	22.00	21.00

Means of eight replications. Means with same letter are not significantly different by Scott-Knott test (P<0.05). [1]Epidemiological variables analysed. DI = disease index, INC = incidence of bacterial wilt, and CI = colonization index.

Table 4. Reaction of tomato rootstocks to bacterial wilt caused by two sequevars of *Ralstonia pseudosolanacearum* strains. Recife, UFRPE, 2017/2018.

Genotype	CRMRS126 (I-17)			CRMRS116 (I-18)		
	DI (%)[1]	INC (%)	CI (%)	DI (%)	INC (%)	CI (%)
BSPE0039	46.87 c	46.87 b	47.25 b	46.25 b	65.62 b	65.62 b
BSPE0041	65.00 b	75.00 a	75.25 a	34.37 c	56.25 b	56.28 b
Defensor	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
Green Barrier	58.75 c	81.25 a	81.25 a	25.00 c	31.25 c	31.25 c
Green Power	68.12 b	84.37 a	84.37 a	23.12 c	50.00 b	50.23 b
Green Rise	61.87 b	71.87 a	72.24 a	82.5 a	90.62 a	90.62 a
Guardião	20.62 c	25.00 c	25.62 c	0.00 d	0.00 d	0.56 d
Hawaii 7996	37.50 c	53.12 b	53.50 b	10.62 d	25.00 c	25.01 c
L 390	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
Muralha	66.87 b	93.75 a	93.87 a	0.00 d	0.00 d	0.53 d
RZ01	68.12 b	90.62 a	90.62 a	30.00 c	37.50 c	38.50 c
RZ02	78.75 b	96.87 a	96.87 a	53.75 b	75.00 b	75.37 b
TD1	75.00 b	90.62 a	90.62 a	93.75 a	96.87 a	96.87 a
Tropithai	85.00 a	87.50 a	87.50 a	83.75 a	96.87 a	96.87 a
Woodstock	54.37 c	78.12 a	78.25 a	0.00 d	0.00 d	0.31 d
Yoshimatsu	81.87 a	87.50 a	87.50 a	40.00 b	62.50 b	62.50 b
CV (%)	41.00	31.00	31.00	43.00	41.00	40.00

Means of eight replications. Means with same letter are not significantly different by Scott-Knott test (P<0.05). [1]Epidemiological variables analysed. DI = disease index, INC = incidence of bacterial wilt, and CI = colonization index.

penetrate the host root systems through wounds and move to the root vessels, reach the xylem, and subsequently spread into the shoot (Digonnet *et al*., 2012). In the xylem, the colonization is critical to disease progress and strains of *R. solanacearum* defective in xylem colonization do not cause wilting in plants (Plener *et al*., 2010). On the other hand, the colonization of the root vascular cylinder is delayed in resistant 'Hawaii 7996' and although bacteria may enter the root vascular tissues, the colonization in the vessel is spatially restricted. These dynamics occur partly due to the ability of the resistant cultivar to restrict bacterial root colonization in space and time (Caldweel *et al*., 2017). This could explain why asymptomatic tomato plants used in this study showed vessels colonized. The presence of the bacterium in the vessels of resistant genotypes explain why grafted plants may show symptoms whenever conditions favor bacterial wilt, such as high temperatures and humidity.

The results obtained in the present study also showed that the level of resistance of the genotypes was specific for each strain, regardless of the species involved, in agreement with the results found in previous studies (Lebeau *et al*., 2011; Lopes *et al*., 2015; Kim *et al*., 2016).

The level of resistance of tomato rootstocks to bacterial wilt is highly related to environmental factors and the high genetic variability of *Ralstonia* species (Rivard *et al.*, 2012; Santiago *et al.*, 2017), especially in Brazil, which is considered an important center of genetic variability for those bacteria (Santiago

Table 5. Reaction of tomato rootstocks to bacterial wilt caused by *Ralstonia* spp. in an area with history of bacterial wilt occurrence. Chã Grande, UFRPE, 2019.

Genotype	AUDPC[1]	DI (%)	INC (%)
Green Rise	7.22 bc[2]	6.25 b	6.25 bc
Guardião	0.00 c	0.00 b	0.00 c
Hawaii 7996	14.45 bc	12.50 b	12.50 bc
L390	89.50 a	56.25 a	56.25 a
Pé Franco (Tomini F1)	105.90 a	68.75 a	68.75 a
TD1	8.10 bc	5.00 b	6.25 bc
Tropithai	19.02 b	16.25 b	18.75 b
Woodstock	0.00 c	0.00 b	0.00 c
Yoshimatsu	0.00 c	0.00 b	0.00 c
CV (%)	49.38	62.43	61.53

[1]AUDPC = area under the disease progress curve, DI = disease index, and INC = incidence of bacterial wilt. [2]Means of four blocks. Means with same letter are not significantly different by LSD test (P<0.05). AUDPC data transformed into $\sqrt{x+0.5}$.

et al., 2017). The combinations of rootstock and graft must be tested according to the climatic conditions and isolated from each region, but the high genetic variability found in populations of the pathogen has hampered the use of resistance sources, since the stability of resistance to bacterial wilt in Solanaceae is highly affected by the variability of the pathogen and by factors linked to the environment (Rivard *et al*., 2012; Ahmed *et al.*, 2013; Albuquerque *et al*., 2021; Santiago *et al*., 2017).

Therefore, based on the different responses presented in the experiments carried out with strains of *R. solanacearum* and *R. pseudosolanacearum*, two rootstocks that showed the highest levels of resistance ('Guardião' and 'Woodstock') and four rootstocks that showed lower levels of resistance ('Green Rise', 'TD1', 'Tropithai', and 'Yoshimatsu') were selected to evaluate the reaction to bacterial wilt in an area with a history of the disease in the Pernambuco state, tropical zone.

Tomato rootstocks reaction to bacterial wilt in an area with history of disease occurrence

The plants of cultivar Tomini F1 grafted into the genotypes 'Green Rise', 'Hawaii 7996', 'Guardião', 'TD'1, 'Tropithai', 'Woodstock', and 'Yoshimatsu', used as rootstocks to protect against bacterial wilt differed significantly, in all studied variables, from 'L390', the susceptible control, and from non-grafted plants of tomato cv. Tomini F1 (Table 5). There were no symptoms of bacterial wilt observed when plants of tomato cv. Tomini F1 were grafted into 'Guardião', 'Woodstock', and 'Yoshimatsu', which did not differ from those grafted onto 'Hawaii 7996', that showed AUDPC, DI, and INC values of 14.45, 12.50%, and 12.50%, respectively. Tomato plants showing symptoms of bacterial wilt randomly selected showed bacterial exudation from the stem in the presence of water. These results are important for disease control, but it may not be compared with experiments carried out in greenhouse because although the presence of different sequevars of RP and RS is known in Chã Grande (Garcia *et al*., 2013, 2014), the identification of the strains causing bacterial wilt was not performed in this experiment. Also, the main objective of this experiment was to analyse the behaviour of the genotypes with the natural inoculum and environmental conditions where the tomato is produced in this mesoregion.

Interestingly, the genotype Yoshimatsu, regarding as susceptible in greenhouse experiments, showed a high resistance in the field experiment. Similarly, in experiments carried out in different regions of the United States, rootstocks grafted with commercial cultivars showed symptoms in greenhouses but were able to produce economically in field tests carried out in areas with soils naturally infested with *Ralstonia* spp. (McAvoy *et al.*, 2012; Rivard *et al.*, 2012). Therefore, the presence of symptoms should not be a determining factor in the selection of rootstocks tolerance, because inoculum doses might be different in the two environments. Thus, it is necessary to carry out complementary field tests to evaluate the productive capacity of the grafted hybrids (McAvoy *et al.*, 2012; Rivard *et al.*, 2012).

'Guardião' and 'Muralha' were found to be resistant in greenhouse experiments, while 'Guardião' was also resistant in an area with historical of the disease, showing potential for use as rootstocks for control of the disease. Therefore, this technique associated with other control measures could contribute to the integrated management of bacterial wilt of Solanaceae in fields with history of the disease, and should not be used isolated, especially when conditions are favourable to the development of bacterial wilt (Marouelli *et al*., 2005; Lopes *et al.*, 2015).

Based on the data obtained, it is concluded that the resistance of rootstocks to bacterial wilt varied according to the strains and the experimental conditions performed, as already described in other studies (Ahmed *et al*., 2013; Albuquerque, *et al*., 2021; Santiago *et al*., 2017). In addition, these results reinforce the importance to evaluate combinations of rootstock and grafting according to the climatic conditions and isolate from each region. In turn, 'Muralha' and 'Guardião' genotypes showed a more stable level of resistance and they could be used as rootstocks in the management of bacterial wilt and as sources of resistance in plant breeding programs.

ACKNOWLEDGEMENTS

We thank the Brazilian Nacional Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq), for the financial support to Géssyka Rodrigues de Albuquerque.

REFERENCES

AHMED, NN; ISLAM, MR; HOSSAIN, MA; HOSSAIN, MM. 2013. Determination of races and biovars of *Ralstonia solanacearum* causing bacterial wilt disease of potato, *Journal of Agricultural Science* 5: 1-8.

ALBUQUERQUE, GMR; SILVA, AMF; SILVA, JR; MELO, EA; MARIANO, RLR; LEMOS, MC; FERRAZ, E; SOUZA, ES. 2021. Sequevar distribution of *Ralstonia* spp. in Solanaceae in the semiarid climate of the Pernambuco state, Brazil. *European Journal of Plant Pathology* 159: 13-25.

BARBOSA, JC; MALDONADO JÚNIOR, W. 2015. AgroEstat: Sistema para análises estatísticas de ensaios agronômicos. Jaboticabal: FUNEP. 396p.

CALDWELL, D; KIM, BS; IYER-PASCUZZI, AS. 2017. *Ralstonia solanacearum* differentially colonizes roots of resistant and susceptible tomato plants. *Phytopathology* 107: 528-536.

COSTA, KDS; SANTOS, AMM; SANTOS, PR; NASCIMENTO, MR; SILVA, AMF; ALBUQUERQUE, GMR; BATISTA, RO; PEREIRA, JWL; CARVALHO FILHO, JLS. 2018. Inheritance of resistance to *Ralstonia pseudosolanacearum* in tomato. *Euphytica* 214: 137.

COSTA, KDS; SANTOS, PR; SANTOS, AMM; SILVA, AMF; CHAGAS, JTB; CARVALHO FILHO, JLS; LIMA, JWP; SILVA, MO; SILVA, JR; MENESES, D. 2019. Genetic control of tomato resistance to *Ralstonia solanacearum*. *Euphytica* 3: 235-246.

DIGONNET, C.; MARTINEZ, Y.; DENANCE, N.; CHASSERAY, M.; DABOS, P.; RANOCHA, P.; MARCO, Y.; JAUNEAU, A.; GOFFNER, D. 2012. Deciphering the route of *Ralstonia solanacearum* colonization in *Arabidopsis thaliana* roots during a compatible interaction: focus at the plant cell wall. *Planta* 236: 1419-1431.

FAO. 2020. Food and Agriculture Organization of the United Nations. Italy. FAOSTAT. Available http://www.fao.org/faostat/en/#data/QC/visualize. Accessed September 14, 2020.

GARCIA AL; LIMA WG; SOUZA EB; MICHEREFF SJ; MARIANO RLR. 2013. Characterization of *ralstonia solanacearum* causing bacterial wilt in bell pepper in the State of Pernambuco, Brazil. *Journal of Plant Pathology* 95: 237-245.

GARCIA AL; SOUZA EB; MARIANO RLR. 2014. Ácidos orgânicos no controle da murcha bacteriana do pimentão. *Revista Brasileira de Ciências Agrárias* 9: 225-230.

GRIMAULT V; ANAIS G; PRIOR P. 1994. Distribution of *Pseudomonas solanacearum* in the stem tissues of tomato plants with different levels of resistance to bacterial wilt. *Plant Pathology* 43: 663-668.

GROVER A; AZMI W; GADEWAR AV; PATTANAYAK D; NAIK PS; SHEKHAWAT GS; CHAKRABARTI SK. 2006. Genotypic diversity in a localized population of *Ralstonia solanacearum* as revealed by random amplified polymorphic DNA markers. *Journal of Applied Microbiology* 101: 798-806.

IBGE. 2020. Instituto Brasileiro de Geografia e Estatística. Brasil. Sistema IBGE de Recuperação Automática – SIDRA: Levantamento sistemático da produção agrícola. Available at: https://sidra.ibge.gov.br/home/lspa. Assessed September 14, 2020.

KELMAN, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology* 44: 693-695.

KIM, SG; HUR, O; RO, N; KO, H; RHEE, J; SUNG, JS; RYU, K; LEE, S; BAEK, HJ. 2016. Evaluation of resistance to *Ralstonia solanacearum* in tomato genetic resources at seedling stage. *Plant Pathology* 32: 58-64.

LEBEAU, A; DAUNAY, MC; FRARRY, A; PALLOIX, A; WAMG, JF; DINTINGER, J; CHIROLEU, F; WICKER, E; PRIOR, P. 2011. Bacterial wilt resistance in tomato, pepper, and eggplant: genetic resourcers respond to diverse strains in the *Ralstonia solanacearum* species complex. *Phytopathology* 101: 154-165.

LOPES, CA. 2009. Murcha bacteriana ou murchadeira: uma inimiga do tomateiro em climas quentes. Available https://www.embrapa.br/busca-de-publicacoes/-/publicacao/782934/murcha-bacteriana-ou-murchadeira-uma-inimiga-do-tomateiro-em-climas-quentes. Accessed March 29, 2020.

LOPES, CA; BOITEUX, LS; ESCHEMBACK, V. 2015. Eficácia relativa de porta-enxertos comerciais de tomateiro no controle da murcha-bacteriana. *Horticultura Brasileira* 33: 125-130.

LOPES, CA; MENDONÇA, JL. 2016. Reação de acessos de jurubeba à murcha bacteriana para uso como porta-enxerto em tomateiro. *Horticultura Brasileira* 34: 356-360.

LOPES, CA; ROSSATO, M. 2018 History and status of selected hosts of the *Ralstonia solanacearum* species complex causing bacterial wilt in Brazil. *Frontiers in Microbiology* 9:1228. doi: 10.3389/fmicb.2018.01228.

MAROUELLI, WA; LOPES, CA; SILVA, WLC. 2005. Incidência de murcha bacteriana em tomate para processamento industrial sob irrigação por gotejamento e aspersão. *Horticultura Brasileira* 23: 320-323.

MCAVOY, T; FREEMAN, JH; RIDEOUT, SL; OLSON, SM; PARET, ML. 2012. Evaluation of grafting using hybrid rootstocks for management of bacterial wilt in field tomato production. *HortScience* 47: 621-625.

MCKINNEY, HH. 1923. Influence of soil, temperature, and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *Journal of Agricultural Research* 26: 195-217.

NAKAHO, K; INOUE, H; TAKAYAMA, T; MIYAGAWA, H. 2004. Distribution and multiplication of *Ralstonia solanacearum* in tomato plants with resistance derived from different origins. *Journal of General Plant Pathology* 70: 115-119.

NIELSEN, LW; HAYNES, FL. 1960. Resistance in *Solanum tuberosum* to *Pseudomonas solanacearum*. *America Potato Journal* 37: 260-267.

PLENER, L; MANFREDI, P; VALLS, M; GENIN, S. 2010. PrhG, a transcriptional regulator responding to growth conditions, is involved in the control of the type III secretion system regulon in *Ralstonia solanacearum*. *Journal of Bacteriology* 192: 1011-1019.

PRIOR, P; AILLOUD, F; DALSING, BL; REMENANT, B; SANCHEZ, B; ALLEN, C. 2016. Genomic and proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three species. *BMC Genomics* 17: 90-117.

PRIOR, P; BART, S; LECLERCQ, S; DARRASSE, A; ANAIS, G. 1996. Resistance to bacterial wilt in tomato as discerned by spread of *Pseudomonas* (*Burholderia*) *solanacearum* in the stem tissues. *Plant Pathology* 45: 720-726.

RIVARD, CL; O´CONNELL, S; PEET, MM; ELKER, RM; LOUWS, FJ. 2012. Grafting tomato to manage bacterial wilt caused by *Ralstonia solanacearum* in the southeastern United States. *Plant Disease* 96: 973-978.

SAFNI, I; CLEENWERCK, I; VOS, P; FEGAN, M; SLY, L; KAPPLER, U. 2014. Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: proposal to emend the descriptions of *R. solanacearum* and *R. syzygii* and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. *syzygii*, *R. solanacearum* phylotype IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. nov. and *R. solanacearum* phylotypes I and III strains as *Ralstonia pseudosolanacearum* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* 64: 3087-3103.

SANTIAGO, TR; LOPES, CA; CAETANO-ANOLLÉS, G; MIZUBUTI, ESG. 2017. Phylotype and sequevar variability of *Ralstonia solanacearum* in Brazil, an ancient center of diversity of the pathogen. *Plant Pathology* 66: 383-392.

SHANER, G; FINNEY, RE. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. *Phytopathology* 67: 1051-1056.

UEHARA, T; NAKAHO, K. 2018. Effects of high grafting on tomato plants infected by *Meloidogyne incognita* and *Ralstonia solanacearum*. *Journal of Phytopathology* 166: 53-58.

WANG, JF; HANSON, P; BARNES, JA. 1998. Worldwide evaluation of an international set of resistance sources to bacterial wilt in tomato. IN: PRIOR, P; ALLEN, C; ELPHINSTONE, J (eds). *Bacterial wilt disease.* Molecular and ecological aspects. Berlin: Springer - INRA. p.269-275.