Reaction of Citrus Genotypes to Citrus Variegated Chlorosis

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ABSTRACT. Citrus variegated chlorosis (CVC), caused by *Xylella fastidiosa*, is one of the main citrus diseases in Brazil. The aim of the current work was to evaluate oranges, mandarins and hybrid varieties which were introduced into Brazil from several countries, for their susceptibility or resistance to CVC, under field conditions, in Bebedouro, SP, Brazil. One hundred and eighty-five citrus genotypes were evaluated. The approach graft method was used for inoculation with previously infected nursery trees as sources of bacteria. The disease was evaluated by a rating scale through visual inspection of symptoms and by PCR testing specific for *X. fastidiosa.* Genotypes that did not show symptoms (although PCR positive) and non-CVC hosts are of interest for their possible use commercially and in programs for genetic improvement.

Index words. Disease resistance, genetic improvement, *Xylella fastidiosa*.

Pera, Natal, Valencia and Hamlin sweet oranges are the main varieties in Brazil and, due to this limited number of varieties, orchards are more vulnerable to new diseases. Citrus variegated chlorosis (CVC), caused by *Xylella fastidiosa*, has been seriously damaging sweet oranges, which are the most economically important type of citrus Brazil (8). The disease spreads in all citrus regions and the incidence and severity of disease has been increasing lately (2).

X. fastidiosa may be transmitted through infected buds or by 11 species of sharpshooters (1). Fruits are small and hard, and have an intense yellow color and are thus useless for fresh consumption or processing (8). CVC also affects some non-commercial tangerines and hybrids (tangors and tangelos), at a lower intensity (6, 7, 8,) and several others evaluated under greenhouse conditions (3).

Some strategies have been adopted for managing the disease, such as planting healthy nursery plants produced in greenhouses with insect-proof screens, removal of infected branches and severely affected trees, and by chemical control of insect vectors (12). In spite of all these procedures, economic losses caused by CVC are very high. These losses could be reduced with resistant varieties. The aim of this work was to evaluate the reaction of 185 citrus genotypes, originally from several different countries, to CVC under field conditions.

MATERIALS AND METHODS

This work was conducted under field conditions in Bebedouro, SP, Brazil (20°53'16"S, 48°28'11"W) which has dry winters and hot and rainy summers. The following genotypes were studied: sour orange, sweet oranges, mandarins, tangors, tangelos and some other hybrids imported by the Citrus Experimental Station of Bebedouro (EECB) from germplasm collections in Italy, Mexico, Portugal, Spain and France with the collaboration of Embrapa Genetic Resources and Biotechnology and Fundecitrus. The genotypes evaluated are shown in Table 1.

In order to obtain nursery trees infected by *X. fastidiosa*, Rangpur lime seedlings were approach-grafted for 30-40 days with adult branches of Pera trees which presented typical CVC foliar and fruit

TABLE 1
CITRUS GENOTYPES EVALUATED AS TO REACTION TO CITRUS VARIEGATED CHLOROSIS

Citrange		
C-13		
C-32		
Clementine		
Caffin SRA 385	2kr Monreal	Commune SRA 92
Commune SRA 85	Commune SRA 88	Oroval Y.45
De Nules VCR	Nules SRA 389	Reina SRA 534
Oroval SRA 335	Ragheb SRA 386	
Tomatera SRA 535	Bruno SRA 531	
Mandarin		
À Peau Lisse SRA 267	Ampefy SRA 495	Ananas SRA 410
Antillaise SRA 497	Beauty of Glen Retreat SRA 261	Burgess SRA 412
C-54-4-4 SRA 337	CAMI	Carv. Vidigueira
Carvalhais	Changsha SRA 413	Ciaculi 60/22a12 Proc. 435/96
East India SRA 414*	Fewtrell SRA 418*	Fuzhu SRA 599
Late Emperor SRA 423	Macaque SRA 426	Malvasio SRA 115
Miúda Proc. 49/97	Natal Tightskin SRA 481*	Ponkan Yoshida SRA 585*
Redskin SRA 428	Rodeking SRA 431*	Setubal
Setubalense Proc. 49/97*	Temple Sue Linda SRA 467*	Vaso
Wallent SRA 438*	Zanzibar SRA 442*	Lebon **SRA 425**
Satsuma		
Kowano SRA 167	Miyagawa SRA 444	Saigon SRA 227
Salzara SRA 341	Unshu SRA 529	
Sweet orange		
Amares*	Amieux Bey-CCC-167	Amieux nuc.-CCC-802
Barile SRA 559*	Barlenn SRA 568*	Belladona
Belladona EECB 165*	Bema IVIA-43-1*	Berna IVIA 43*
Berna Peret IVIA 336*	Biondo Corigliano I*	Biondo Di Caccia*
Boukhobza SRA 569*	Cadenera Punchosa (Campo)*	Callao-CCC-628
Caprichosa-CCC-029	Casa Grande SRA 183*	Castellana IVIA-64-3*
China-CCC-030	China*	China SRA 547*
Comum Resistente al frio-CCC-602	Comuna IVIA-l05*	Convento*
Crescent-CCC-584	D. João 107	D. João Proc. 49/97*
Damasco-CCC-375	Doblefina*	Doce Espanha
Evora S/S	Fraga Proc. 49/97*	Fukuhara SRA 561*
Fullamenuda IVIA 92*	Grada	Hall SRA 394*
Jaffa*	Joppa nuc.-CCC-764	Maçã
Maçã EECB 182*	Murtera-IVIA-54*	Navelina ISA 315*
Navelina SRA 332*	Newhall Navel SRA 343*	Ovale
Ovale Mut Proc. 435/96*	Pala*	Pardilhó
Pêra IAC (testemunha)	Pêra Vidigueira	Pera Vidigueira (Sr. Antunes)*
Petit Pierre ½ sanguine SRA 570*	Portela	Prata Proc. 49/97*
Prata da Ponte*	Prata Lima	Premier SRA 510*
Queen nuc.-CCC-820	R.A.H.	Rohde Red nuc.-CCC-423
Rotuna SRA 511*	Sanford SRA 404*	Sanguinea 66/SsaJl2 Proc. 435/96*
Seleta tardia	Seleta Tardia EECB 172*	Skaggs Bonanza Navel SRA 202*
Sokotoro SRA 407*	Sweet SRA 50*	Tetouan-CCC-171
Torregrossa IVIA 103-6*	Tua (Sr. Mamede)*	TUA*
TUA Grauda 1*	TUA Ponte*	TUA S/S*
Vale dos Besteiros*	Valencia Bertoni-CCC-413	Valencia Campbell*
Valencia cv-148-CCC-685	Valencia cv-25-CCC-683	Valencia cv-27-CCC-687

(*) Genotypes evaluated in plots 1 and 2.

TABLE 1 (CONTINUED)
CITRUS GENOTYPES EVALUATED AS TO REACTION TO CITRUS VARIEGATED CHLOROSIS

Valencia cv-59-CCC-684	Valencia Huber-CCC-604	Valencia Late Burjasot IVIA-35-2*
Valencia Muller-CCC-716	Valencia Rohde Red SRA 360*	Valência Temprana IVIA 25*
Vera IVIA-97*	Verde de Espanha	Yoshida Navel SRA 558*
Zoumi-CCC-172		
Sour orange		
Azeda Beja*		
Tangelo		
Encore SRA 190	Fortune SRA 31	Mapo
Nova IVIA-74-7	Nova IVIA-86-2	Page SRA 159
Tangelo-Allspice SRA 327	Tangelo-Guyane SRA 448	Tangelo-Mapo SRA 450
Tangelo-Nocatee SRA 452	Tangelo-Nova SRA 158	Tangelo-Thornton Vero SRA 460
Tangor		
Clemelin IVIA 355	Ellendale-CCC-411	OMO 12
OMO 13	OMO 14	OMO 15
OMO 16	OMO 17	OMO 20
OMO 22	OMO 24	OMO 27
OMO 28	OMO 29	OMO 30
OMO 31	OTA 11	OTA 12
OTA 14	OTA 15	OTA 17
OTA 23	OTA 27	OTA 28
OTA 29	OTA 32	OTA 33
OTA 34	Dweet IVIA-C-165	H-56 SRA 465
Other		
Mineola tangelo × trifoliate orange		

(*) Genotypes evaluated in plots 1 and 2.

symptoms and which had been confirmed as infected through pathogenicity tests. These infected nursery trees were then approach grafted onto the varieties under study, and were kept on their own root systems until the grafts had taken. After this they were cut off from their roots.

The work was conducted in two plots: 1) Three nursery trees, for each of the 184 genotypes were planted at 7 × 3 m spacing, in February, 2000. One tree of each variety was inoculated 11 mo later as described above; 2) Eight trees from 67 of the varieties (57 sweet oranges, one sour orange and eight mandarins), selected from the first plot based on their horticultural characteristics, as well as Pera sweet orange as a control, were planted at 6 × 2 m spacing in four blocks with two plants of each per block, in April, 2001, and inoculated 9 mo after planting as described above.

The presence of typical symptoms of CVC was evaluated visually twice a year by rating on a scale of [0] no symptoms, [1] plant with some symptomatic leaves (up to one branch with symptoms), [2] 50% of the plant with symptoms, and [3] 100% of the plant with symptoms. The evaluations were ended 43 mo after inoculation in plot 1 and after 27 mo in plot 2.

For PCR, specific *X. fastidiosa* primers CVC1, RST 31 and RST 33 were used, with DNA extraction (14), and quantification (13) performed as described. PCR testing was conducted for oranges and tangerines in plots 1 and 2 in 2002 and 2003, respectively. Genotypes were evaluated as symptomatic hosts when they presented visual symptoms in leaves or fruits, as non-symptomatic hosts when they were PCR positive, and as non-hosts when they were PCR negative.

RESULTS AND DISCUSSION

Reaction of the genotypes under study varied according to species. Among the sweet oranges genotypes, only Navelina ISA 315 and Newhall Navel SRA 343 did not show any symptoms, although they were positive with PCR (Table 2). Other promising genotypes were Callao-CCC-628, Crescent-CCC-584, Damasco-CCC-375 and Queen nuc.-CCC-820 which were both non-symptomatic and PCR negative (Table 3), although we note that the PCR results are preliminary. The results obtained confirmed the great susceptibility of sweet orange as reported previously in field (7, 9), nursery and greenhouse conditions (10, 15). If the horticultural quality of these varieties is suitable, they could be commercially used.

In a previous study conducted under greenhouse conditions, the absence of symptoms was reported in several mandarins and mandarin hybrids despite positive results of specific pathogenicity tests (3). Except for Cami which developed symptoms, all the mandarin genotypes were either non-symptomatic hosts (Table 2) or non-hosts (Table 3). Previous studies reported the occurrence of leafy symptoms in the varieties Carvalhais, Wilking, Tankan, Bower, Clem × Honey and Emperor (7, 8). Nevertheless, the results of this work were different for Carvalhais mandarin, which was a non-symptomatic host. The Late Emperor variety was found to be a non-symptomatic host, although its parent, Emperor (4), has been reported to be a symptomatic host (7, 8).

Tangelos were of interest since some appear to be non-symptomatic hosts. Orlando tangelo has been reported to be a non-symptomatic host (11), while symptoms have been found in Page, Suwanee and Williams (6, 7, 8), although we found Page to be a non-symptomatic host.

Dweet and Clemelin IVIA 355 tangors displayed symptoms. These results are in accordance with the ones obtained in other investigations (7, 8). Temple Sue Linda tangor did not show any symptoms in this work, a result differing from others reports (7, 8), assuming that the same variety was used. Ellendale tangor presented no symptoms, but its host status was not evaluated by PCR.

TABLE 2
GENOTYPES OF CITRUS CLASSIFIED AS NON-SYMPTOMATIC HOSTS

Group	Genotypes non-symptomatic hosts
Sweet Orange	Navelina ISA 315*, Newhall Navel SRA 343*
Sour Orange	Beja*
Mandarin	À Peau Lisse SRA 267, Ampefy SRA 495, Ananas SRA 410, Antillaise SRA 497, Beuty Of Glen Retreat SRA 261, Burgess SRA 412, C-54-4-4 SRA 337, Carv. Vidigueira, Carvalhais, Changsha SRA 413, Ciaculi 60/22a/2 Proc. 435/96, East India SRA 414*, Fewtrell SRA 418*, Fuzhu SRA 599, Late Emperor SRA 423, Lebon SRA 425, Macaque SRA 426, Malvasio SRA 115, Miúda Proc. 49/97, Natal Tightskin SRA 481*, Ponkan Yoshida SRA 585*, Redskin SRA 428, Rodeking SRA 431*, Temple Sue Linda SRA 467*, Vaso, Wallent SRA 438*, Zanzibar SRA 442*
Clementine	2kr Monreal, Bruno SRA 531, Commune SRA 85, Commune SRA 88, Commune SRA 92, Nules SRA 389, Oroval Y.45, Oroval SRA 335, Ragheb SRA 386, Tomatera SRA 535
Tangelo	Allspice SRA 327, Encore SRA 190, Fortune SRA 31, Guyane SRA 448, Mapo, Nocatee SRA 452, Nova IVIA-74-7, Nova IVIA-86-2, Nova SRA 158, Page SRA 159, Thornton Vero SRA 460
Tangor	H-56 SRA 465, OTA 28
Satsuma	Kowano SRA 167, Miyagawa SRA 444, Saigon SRA 227, Salzara SRA 341, Unshu SRA 529

*Genotypes evaluated in plots 1 and 2.

TABLE 3
GENOTYPES OF CITRUS CLASSIFIED AS NON-HOSTS OF *XYLELLA FASTIDIOSA*

Group	Non-host Genotypes
Sweet Orange	Callao-CCC-628, Crescent-CCC-584, Damasco-CCC-375, Queen nuc.-CCC-820
Clementine	Caffin SRA 385, De Nules VCR
Tangelo	Mapo SRA 450
Tangor	OMO 12, OMO 15, OMO 16, OMO 17, OMO 20, OMO 22, OMO 24, OMO 27, OMO 28, OMO 29, OMO 31, OTA 11, OTA 12, OTA 14 , OTA 15, OTA 17, OTA 23, OTA 27, OTA 29, OTA 33, OTA 34
Citrange	C-13, C-32
Other	Mineola × trifoliate orange

There are still no records of plantings of commercial mandarin varieties affected by the disease or performing as inoculum source for susceptible cultivars (5).

The results show the higher susceptibility to CVC of sweet oranges. Genotypes classified as non-symptomatic hosts and non hosts may have direct commercial use as well as in programs for genetic improvement for CVC resistance.

ACKNOWLEDGMENTS

Special thanks to 'Fundação de Amparo a Pesquisa do Estado de São Paulo-FAPESP (00/08692-9)' and to 'Fundo de Defesa da Citricultura—support in order to accomplish this work, and to Laboratório de Bioquímica de Microorganismos de Plantas, FCAV—UNESP for helpful assistance with laboratory work.

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