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Biovar-specific and broad-spectrum sources of resistance to bacterial wilt (*Ralstonia solanacearum*) in *Capsicum*

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ABSTRACT *- No report has so far investigated either the biovar-specific or broad-spectrum reaction to* Ralstonia solanacearum *(RS) in* Capsicum *germplasm. Twenty-three accessions described as resistant to RS in different countries were challenged with Brazilian biovar I and biovar III isolates. Differences were observed among accessions and within biovar III isolates. In addition, isolates of biovar III were more aggressive than biovar I, reinforcing field observations which indicated that RS biovar III is predominant and more virulent and aggressive than biovar I isolates on* Capsicum. *Accessions previously reported as extremely resistant to RS in Asia exhibited slight wilt symptoms when challenged with Brazilian isolates. However, the high resistance levels in the inbred lines 'MC-4', 'PBC 631', 'PBC 066', 'PBC 1347', and 'PBC 473' make these accessions suitable for breeding programs aiming to develop pungent and/or sweet cultivars with a stable, broad-spectrum RS-resistance.*

Key words: biovar, *Capsicum*, pepper, *Pseudomonas solanacearum*, resistance.

INTRODUCTION

Ralstonia solanacearum E.F. Smith 1896 (Yabuuchi et al. 1995) (RS) is a gram-negative, soil-borne pathogen that causes bacterial wilt (BW), a disease affecting more than 50 plant families (Hayward 1994a). This disease is economically important for several vegetable crops including members of the Solanaceae family such as tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), eggplant (*Solanum melongena* L.), and sweet pepper (*Capsicum annuum* L.) (Hartman and Elphinstone 1994). BW has worldwide distribution but is more severe in hot-wet tropical and subtropical regions where the environmental conditions favor year-round survival and facilitate spread and colonization of a broad range of natural hosts. The employment of resistant cultivars is one of the few strategies offering low-cost and efficient control of BW in solanaceous crops (Peter et al. 1993, Hartman and Elphinstone 1994, Wang et al. 2000).

The typical wilt symptoms induced by RS are result of the endophytic growth and selective secretion of large amounts of bacterial polysaccharides that block the xylem of the infected plants (Buddenhagen and Kelman 1964). So far, the virulence profile of RS isolates has been primarily grouped into five races according to their ability to infect members of distinct groups of plant taxa under certain environmental conditions (Hayward 1994a). Molecular and biochemical characterization and/or classification systems for RS isolates have been reported in literature. One of them is the widely

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used biovar system that discriminates isolates according to some phenotypic properties such as the capability of oxidizing disaccharides, utilizing hexose alcohols, and producing gas from nitrate (Hayward 1991, 1994b). The biovar profiling was initially employed as an additional laboratory trial to group RS isolates (Hayward 1994a, b). However, the biovar classification system has somewhat unexpectedly been associated with some peculiar characteristics such as membrane protein patterns (Dristig and Dianese 1990), ecological niches, host range, and geographical distribution of RS isolates (Prior et al. 1990, Hayward 1994b, Lopes et al. 1998, Coelho Netto et al. 2003).

Approximately 13000 hectares per year are planted with hot and sweet pepper (*Capsicum* spp.) in Brazil. Over the years, sweet pepper has been mostly cultivated in open fields. However, in order to attend the increasing demand of cosmetically-oriented consumers, cultivation under plastic cover has dramatically increased in the last decade. Drip irrigation and black plastic mulching (prevalent in this production system) have significantly increased the incidence and economic losses caused by soil-borne diseases, including BW incited by RS race 1 (biovars I and III) (Quezado-Soares and Lopes 1995). The evaluation of a collection of RS isolates obtained from infected plants under field conditions in Brazil stated the somewhat unexpected trend that BW in *Capsicum* is primarily caused by biovar III but mostly associated to biovar I in tomatoes (Takatsu and Lopes 1997, Coelho Neto et al. 2003). In addition, field observations indicated that biovar III isolates are apparently more virulent and aggressive for *Capsicum* species by inciting severe wilt and vascular discoloration, whereas biovar I isolates induce less severe symptoms and are mostly limited to plant dwarfism and partial and/or temporary wilting, even under disease-favorable conditions. In view of the highly variable nature of RS variations may be found within these trends in symptom expression.

Several screening trials looking for resistance to RS race 1 in *Capsicum* species have been reported (Peter et al. 1993, Hartman and Elphinstone 1994, Matsunaga and Monma 1999). More recently, Wang and Berke (1997) evaluated the stability of BW resistance in 17 *C. annuum* accessions maintained at the Asian Vegetable Research and Development Center (AVRDC) by inoculating the plants with a biovar III RS isolate. The frequency of affected plants of the selected genotypes varied from 0 to 39%, compared to 96% of the plants with wilt symptoms in the susceptible check. In Brazil, sources of BW-resistance were first sought in hot pepper (*C. chinense*) germplasm (Cheng 1989). Later, Matos et al. (1990) screened 45 *Capsicum* accessions, detecting high resistance levels in three *C. annuum* lines viz. ‘MC-4’ (‘CNPH 143’), ‘MC-5’ (‘CNPH 144’), and ‘HC-10’ (‘CNPH 145’). However, no extensive investigation dealing with the reaction of *Capsicum* species germplasm against distinct biovar I and biovar III isolates has been carried out so far. Objective of this study was to report the reaction of *Capsicum* accessions (23 previously selected as BW-resistant and two susceptible controls) to *R. solanacearum* biovar I or III isolates. This information would be important to define the most appropriate resistance sources and breeding strategies aiming to develop *Capsicum* cultivars with a stable, broad-spectrum BW-resistance.

MATERIAL AND METHODS

Capsicum germplasm

The evaluated germplasm consisted of nine accessions kindly provided by JF Wang, identified as BW-resistant at AVRDC, and 14 accessions identified as resistant in a previous screening of 241 entries of *Capsicum* spp. carried out at Embrapa Hortaliças (Lopes and Quezado-Soares 2001). The *Capsicum* accessions evaluated in the present work are listed in Table 1.

Isolates of *Ralstonia solanacearum* biovars I and III

The six isolates of *R. solanacearum* used in this study were obtained from bell pepper and were chosen for their virulence expressed in susceptible tomato and pepper seedlings upon needle inoculation. The biovar I isolates were named RS 008 (collected in Leticia, Colombia); RS 190 (Tabatinga county, Brasília, Brazil); and RS 201 (Taquara county, Brasília, Brazil). The biovar III isolates were named RS 034 (collected in Boca do Acre, Acre State, Brazil); RS 059 (Rio Preto da Eva, Amazonas State, Brazil); and RS 063 (Manaus, Amazonas State, Brazil). These bacterial isolates from a collection maintained at -80 ^{o}C in 10% glycerol to preserve virulence were recovered in tetrazolium medium (Kelman 1954). Inoculum was prepared from virulent-like colonies restreaked on the same medium but devoid of tretrazolium chloride.

Germplasm evaluation

The experiments were carried out in a greenhouse at an air temperature between 20 and 40 °C at CNPH (Embrapa Hortaliças) in Brasília, Federal District, Brazil. The genotypes were sown onto styrofoam trays with 128 cells filled with sterile substrate. When the plants had fully opened the first two pairs of true leaves they were removed from the cells with a gentle jet of water to preserve root integrity. They were then dipped

Table 1. *Capsicum* accessions screened for resistance to *Ralstonia solanacearum* biovar I and biovar III isolates

CNPH Code[1]	AVRDC code	Line designation	Origin	Species	Type
CNPH 26	-	'BGH 3056'	Brazil	*C. annuum*	chilli
CNPH 143	-	'MC4(S)'	Malaysia	*C. annuum*	chilli
CNPH 148	-	'PM 702'	Mexico	*C. annuum*	chilli
CNPH 192	-	'Magda'	Brazil	*C. annuum*	sweet
CNPH 281	-	'Pimenta de cheiro'	Brazil	*C. chinense*	chilli
CNPH 283	-	'Cambuci'	Brazil	*C. baccatum*	chilli
CNPH 644	-	'Cseresznypaprika'	Hungria	*C. annuum*	chilli
CNPH 682	-	'PI 163201'	India	*C. annuum*	chilli
CNPH 746	-	'PI 370373'	Yugoslavia	*C. annuum*	sweet
CNPH 920	-	'Aji Rojo'	Chile	*C. annuum*	chilli
CNPH 970	-	'Pimenta de cheiro'	Brazil	*C. chinense*	chilli
CNPH 988	-	'Line 20-6'	USA	*C. annuum*	chilli
CNPH 1397	-	'Pimenta 6B'	Brazil	*C. baccatum*	chilli
CNPH 2780	-	'LV 1583'	Indonesia	*C. annuum*	chilli
CNPH 2783	-	'Hot Beauty'	Taiwan	*C. annuum*	chilli
CNPH 2793	-	'LV 2319'	Indonesia	*C. annuum*	chilli
CNPH 3350	PBC066	'MC4'	Malaysia	*C. annuum*	chilli
CNPH 3351	PBC204	'Lang Kap'	Malaysia	*C. annuum*	chilli
CNPH 3352	PBC375	'Paris Minyak'	Indonesia	*C. annuum*	chilli
CNPH 3353	PBC385	'PBC385'	Malaysia	*C. annuum*	chilli
CNPH 3354	PBC473	'PBC473'	Indonesia	*C. annuum*	chilli
CNPH 3355	PBC535	'All Season'	Philippines	*C. annuum*	chilli
CNPH 3356	PBC631	'CA8'	Sri Lanka	*C. annuum*	sweet
CNPH 3357	PBC743	'Chinda 2'	Thailand	*C. annuum*	chilli
CNPH 3358	PBC1347	'Ri-26(17)'	Malaysia	*C. annuum*	chilli

[1]Accessions are listed according to the number of the Embrapa Hortaliças (CNPH) germplasm collection code, followed (when available) by the Asian Vegetable Research and Development Center (AVRDC) code

for 1 minute into a bacterial suspension adjusted to approximately 10^8 cfu mL^{-1}. After inoculation, the plantlets were transplanted to 0.7 kg plastic pots with sterile substrate Plantmax, two plants a pot, and replaced in the greenhouse. The experimental plots consisted of three pots in a randomized block design in three replications. The disease was assessed 12 days after inoculation according to a modified scale of Winstead and Kelman (1952) with the following numerical grades: 0 = no symptoms; 1 = less than 25% of the plant wilted; 2 = more than 25% to 50% of plant wilted; 3 = more than 50% of wilting, but plant still alive; and 4 = dead plant. Individual readings were converted to indices by the summation of the number of plants in each grade multiplied by the respective grade, divided by the maximum numerical grade for the number of evaluated plants and then multiplied by 100. Since the distribution of the disease values was not normal but skewed to the higher resistance levels (due to previous selection) the genotypes were compared with the susceptible ('Magda') and with the resistant ('MC-4') checks by Dunnett's test (Montgomery 1984) as implemented in SAS software (SAS Institute 1989).

RESULTS AND DISCUSSION

Differences were observed among accessions, biovars, and isolates within biovar III (Table 2). Isolates of biovar I provoked hardly any wilt symptoms in the tested genotypes, being only weakly virulent for 'Aji Rojo' ('CNPH 920') and the susceptible control 'Magda' (CNPH 192). The lines 'CNPH 970' (*C. chinense*) and 'CNPH 1397' (*C. baccatum*) had biovar I-specific resistance but were highly susceptible to at least two biovar III isolates. Virulence levels were only variable within the group of biovar III isolates and differences among accessions were observed for all three isolates, especially for isolate RS 059, which was the most virulent in absolute terms (Table 2). Specific responses to certain biovar III isolates were also observed in several *Capsicum* lines with a much clearer expression in accession 'CNPH 3355' for isolate RS 059 (Table 2). The *C. annuum* line 'CNPH 143', a selection from 'MC-4' ('CNPH 3350') introduced from AVRDC, was ranked amongst the most resistant genotypes, showing only slight symptoms even when challenged with the most virulent isolates.

Our results showing biovar-specific response of *Capsicum* germplasm are in agreement with the initial observations of Takatsu and Lopes (1997) in Brazil where biovar III isolates were predominantly isolated from *Capsicum* whereas biovar I isolates were predominant in tomatoes. This partly explains why BW is important for tomatoes but not important for *Capsicum* species in Florida, where only biovar I isolates were found (Momol et al. 2002). This association is very often observed in isolations from environments where both vegetable crops and both biovars are coexisting, as in the Amazon Basin region (Coelho Netto et al. 2003). This phenomenon deserves further research, since this trend was less evident in a previous study (Quezado-Soares and Lopes 1995). It would be interesting to check whether there is a genetic association between factors determining the biovar profile with either regulatory genes that are required for root infection (Vasse et al. 2000) or genes that modulate the RS host-specificity and aggressiveness (Lavie et al. 2002).

The *C. annuum* lines derived from 'MC-4' were amongst the most resistant genotypes, showing only slight symptoms even when challenged with the most virulent isolates. It is interesting to point out that after inoculation

Table 2. Disease index of selected *Capsicum* genotypes inoculated with *Ralstonia solanacearum* biovar I (isolates RS 008, RS 190 and RS 201) and biovar III (isolates RS 034, RS059 and RS063)

CNPH number	Line designation	Biovar I isolates			Biovar III isolates		
		RS 008	RS 190	RS 201	RS 034	RS 059	RS 063
CNPH 143[1]	'MC-4'	0.0	0.0	0.0	0.0	18.3	0.0
CNPH 192[2]	'Magda'	39.2	42.5	34.2	80.8	100.0	48.3
CNPH 3354	PBC 473	0.0	0.0	0.0	0.0*	0.0*	0.0*
CNPH 3358	PBC 1347	0.0	0.0	0.0	0.0*	0.0*	0.0*
CNPH 3356	PBC 631	0.0	0.0	0.0	0.0*	3.3*	0.0*
CNPH 3352	PBC 375	0.0	0.0	0.0	15.8*+	7.5*	0.0*
CNPH 746	-	0.0	0.0	0.0	7.5*	10.8*	6.7*
CNPH 3353	PBC 385	0.0	0.0	0.0	7.5*	25.8*	0.0*
CNPH 3350	PBC 066	0.0	0.0	0.0	10.8*	24.2*	0.0*
CNPH 3357	PBC 743	0.0	0.0	0.0	16.7*	14.2*	0.0*
CNPH 2780	-	0.0	0.0	0.0	13.3*+	27.5*	3.3*
CNPH 2783	-	0.0	0.0	0.0	36.7*+	31.7*	0.0*
CNPH 3351	PBC 204	0.0	0.0	0.0	10.0*	52.5*+	10.8*
CNPH 3355	PBC 535	0.0	0.0	0.0	2.5*	74.2*+	0.0*
CNPH 2793	-	0.0	0.0	0.0	23.3*+	80.8*+	0.0*
CNPH 281	-	0.0	0.0	0.0	25.8*+	63.3*+	25.8+
CNPH 148	-	0.0	0.0	0.0	36.7*+	83.3 +	4.2*
CNPH 283	-	0.0	0.0	0.0	44.2*+	90.0 +	0.0*
CNPH 682	-	0.0	0.0	0.0	29.2*+	100.0 +	0.0*
CNPH 1397	-	0.0	0.0	0.0	54.2*+	60.0*+	18.3*
CNPH 644	-	0.0	0.0	0.0	49.2*+	89.2 +	0.0*
CNPH 26	-	0.0	0.0	0.0	68.3+	74.2*+	0.0*
CNPH 988	-	0.0	0.0	0.0	62.5+	80.0*+	5.0*
CNPH 920	-	28.3	29.2	30.0	30.0*+	100.0 +	33.3 +
CNPH 970	-	0.0	0.0	0.0	85.0+	76.4*+	49.2 +

[1]Resistant control used for comparison with all other accessions
[2]Susceptible control used for comparison with all other accessions
* Different from the susceptible control 'Magda' (Dunnett 5%)
+ Different from the resistant control 'MC-4' (Dunnett 5%)
The disease index was calculated based on evaluation of individual plants 12 days after inoculation

with Brazilian isolates, line ‘CNPH 143’, a selection of the original ‘MC-4’ (‘CNPH 3350’) population presented a slight, even though not statistically significant improvement in the resistance levels of this line (Table 2). Our results confirm the high levels of resistance stability of ‘MC-4’ to RS isolates from Brazil, South Korea, and Taiwan (Quezado-Soares and Lopes 1995, Wang and Berke 1997, Kim-Byung et al. 1998). The resistance of nine *Capsicum* genotypes selected at AVRDC for BW-resistance in Asia was confirmed when challenged with Brazilian isolates of *R. solanacearum*. All of these genotypes were ranked among the 13 most resistant materials (Table 2). It must also be pointed out that ‘PBC 473’ and ‘PBC 1347’ were the only genotypes that presented total absence of disease throughout the entire experiment (Table 2). These two genotypes showed, respectively, 2.7% and 6.1% of wilted plants in a trial conducted in Taiwan (Wang and Berke 1997). On the other hand, the only genotype completely devoid of symptoms in the Taiwan trial ‘PBC 385’ was slightly affected by isolates RS 034 and RS 059. This result supports Wang and Berke (1997) in their view that BW-resistance in *Capsicum* is more likely related to the control of bacterial growth in the plant xylem and not to a complete resistance based on restriction to tissue penetration and colonization. This response in *Capsicum* was referred to by these authors as “tolerance”. The high resistance levels of ‘PBC 066’ (‘MC-4’) and ‘PBC 631’ in Korea (Kim-Byung et al. 1998) and of ‘PBC 066’ and ‘PBC 1347’ in Taiwan (Wang and Berke 1997) were confirmed in Brazil. These genotypes, together with ‘PBC 473’, are suitable as sources of broad-spectrum resistance to BW for breeding programs. Since ‘PBC 631’ belongs to the “sweet” group, in distinction to all the others which are of the “chilli” group (Wang and Berke 1997), it might be of particular interest for breeding programs as a source to fasten incorporation of BW-resistance in sweet pepper cultivars for fresh market consumption.

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Fontes de resistência de amplo espectro e biovar-específica à *Ralstonia solanacearum* em *Capsicum*

RESUMO - *Não existem relatos fazendo referência a fontes de resistência de amplo espectro ou biovar-específica à* Ralstonia solanacearum *(RS) em germoplasma de* Capsicum. *Vinte e três acessos descritos como resistentes à RS em diferentes países foram inoculados com isolados brasileiros de RS biovar I e biovar III. Diferenças foram observadas entre acessos e dentro dos isolados de biovar III. Os isolados de biovar III foram mais agressivos que isolados de biovar I, reforçando observações de campo que sugerem que o biovar III é predominante, mais virulento e agressivo em* Capsicum. *Acessos previamente identificados com extrema resistência a isolados asiáticos exibiram sintomas leves com isolados brasileiros. No entanto, os elevados níveis de resistência observados nos acessos ‘MC-4’, ‘PBC 631’, ‘PBC 066’, ‘PBC 1347’ e ‘PBC 473’ indicam que estes podem representar valiosas fontes para programas de melhoramento visando o desenvolvimento de linhagens doces e/ou picantes com resistência estável e de amplo espectro à RS.*

Palavras-chave: biovar, *Capsicum*, pimenta, *Pseudomonas solanacearum*, resistência.

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