

Multiple genetic resistances in *Capsicum* spp

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ABSTRACT. This study aimed to identify *Capsicum* genotypes with resistance to bacterial spot (BS), anthracnose and Pepper yellow mosaic virus (PepYMV). Fifty-four genotypes of *Capsicum* spp were evaluated. Resistance reaction against BS was evaluated using three replicates, testing hypersensitivity and quantitative resistance in leaves. After evaluation, inoculated leaves were detached from the plants, being then cultivated until reproductive stage for evaluations anthracnose resistance in immature and mature fruit, totalizing 18 fruits per genotype. For PepYMV resistance was performed with five replications. Each genotype reaction was evaluated by a scoring scale, using the area under the disease progress curve for each pathosystem, and incubation period for the three systems. The latent period was evaluated only for the pathosystem *Capsicum-Colletotrichum gloeosporioides*. Means were grouped by the Scott-Knott test. Measures of dissimilarity matrix among the genotypes were obtained by Gower's algorithm and the grouping was obtained by the UPGMA clustering method. The accessions belonging to the *Capsicum frutescens* were the most susceptible to the three diseases. At least one genotype of *Capsicum baccatum* var. *pendulum*, *Capsicum annuum*, and *Capsicum chinense* showed resistance potential to BS and PepYMV, for use in breeding

programs. The accession UENF 1381 (*C. annuum*) was resistant to the three pathogens.

Key words: *Xanthomonas euvesicatoria*; *Colletotrichum gloeosporioides*; Chili pepper; Pepper yellow mosaic virus

INTRODUCTION

In recent years, consumption and production of cultivars of plants of the genus *Capsicum* have been growing worldwide. Chilies and sweet peppers are in the fourth and eighth positions regarding in growing area of vegetable production around the world (FAOSTAT, 2012). In 2013, it reached a world production of 31.14 million tons within nearly two million hectares (FAOSTAT, 2013). Such demand is increasing given a widening of uses. Traditionally, this plant is used for color, flavor, and aroma in food preparations. Lately, sweet peppers and chilies have been increasingly used for ornamental purposes, as well as in food and pharmaceutical industries. A few substances extracted from *Capsicum* fruit may induce formation and improve connections between neurons such as epigenin (Souza et al., 2015), besides being applied to cancer treatments as capsaicin (Clark and Lee, 2016), among other uses. These applications provide more visibility to this plant genus, boosting the development of new varieties to meet all different demands.

Diseases compose the main limiting factor of *Capsicum* growing. These include bacterial spot (*Xanthomonas* spp), anthracnose (*Colletotrichum* spp), and Pepper yellow mosaic virus (PepYMV) (Riva-Souza et al., 2009; Bento et al., 2013; Diao et al., 2017).

In Brazil, bacterial spot (BS) is mostly caused by *Xanthomonas euvesicatoria* (Areas et al., 2015). This disease is difficult to control in the field, causing great losses to the farmers. In addition, it is highly destructive, being favored by high temperatures and humidity (Stall et al., 2009). As prevention and control measures, it is recommended using healthy seeds and seedlings, removing crop remains, besides using crop rotation and resistant cultivars (Pinto et al., 2011). The latter is the most effective among the control measures. Several studies have aimed to understand the interaction between *Capsicum* and *Xanthomonas* (Jones et al., 2004; Vallejos et al., 2010; Ryan et al., 2011; Potnis et al., 2012), seeking for resistance sources (Costa et al., 2002; Riva-Souza et al., 2009; Moreira et al., 2013) by characterization of the gene expression (Jones et al., 1998; Riva et al., 2004b; Kiss et al., 2016), genetic inheritance (Riva et al., 2004a), developing molecular markers for resistance genes (Holdsworth and Mazourek, 2015), and use of transgenics (Zipfel and Jones, 2015). Despite these studies, few pure line cultivars that are resistant to BS are available in the seed market. Pimenta et al. (2016) developed and protected two cultivars of pepper (*Capsicum annuum* var. *annuum*) with BS resistance, being the first species protected in Brazil.

Another important disease affecting plants of the *Capsicum* genus is anthracnosis, which is caused by a complex of *Colletotrichum* species. At least 15 species of this genus were identified from a collection of 1285 isolates (Diao et al., 2017), among which are *C. fiorinae*, *C. fructicola*, *C. gloeosporioides*, *C. scovillei*, and *C. truncatum* (syn. *C. capsici*). In this identification study, seven species were reported for the first time in chili peppers (*C. aenigma*, *C. cliviae*, *C. endophytica*, *C. hymenocallidis*, *C. incanum*, *C. karstii*, and *C. viniferum*); and other three new ones were recognized and described (*C. conoides*, *C. grossum*, and *C. liaoningense*). Anthracnose is caused by a cosmopolitan pathogen; it can damage an

entire plant, being the fruit mostly impacted in both pre- and post-harvest. This disease control is regularly made through fungicide spraying; however, it oftentimes has a slow efficiency depending on environmental conditions, such as temperatures and high humidity and/ or frequent and severe rainfalls (Haddad et al., 2003). In this case, plant-breeding programs search for cultivars showing steady resistance and against broad-spectrum diseases (Reis et al., 2009).

PepYMV is a member of the *Potyvirus* genus and occurs naturally in crops, provoking great losses since it is easily spread. The main symptoms are leaf twisting, mosaic development, dwarfism, and fruit deformity (Maciel-Zambolim et al., 2004; Bento et al., 2013). Seven genic loci have already been identified in *pvr* series imparting resistance to the *Potyvirus* genus in *Capsicum* plants. Among them, five are recessive (*pvr1*, *pvr2*, *pvr3*, *pvr5*, and *pvr6*) and two are dominant (*Pvr4* and *Pvr7*) (Parrela et al., 2002; Nogueira et al., 2012). Identifying and manipulating these or other new resistance genes have great importance for the development of new resistant cultivars. According to Lucinda et al. (2012), PepYMV resistant cultivars are mandatory in some regions of Brazil because of an intense occurrence of such virus. When evaluating 127 accessions of *Capsicum* spp for resistance to PepYMV, Bento et al. (2009) identified nine resistant genotypes, two of them from *C. baccatum* var. *pendulum* and seven of *C. chinense*.

Given the current world stage where a paradoxical search for healthier diets together with an unrestrained use of pesticides to control crop diseases, new pepper cultivars with multiple resistance to bacterial spot, anthracnose, and PepYMV become such a contribution for farmers, consumers and seed traders since it may reduce production costs as well as increase crop environmental sustainability.

Therefore, this study aimed to identify genotypes with multiple resistance to BS, anthracnose, and PepYMV, which will be useful for breeding programs on peppers of the genus *Capsicum* spp aiming to farm sustainability.

MATERIAL AND METHODS

Genotypes

Fifty-four genotypes were evaluated, comprising 39 accessions from the UENF germplasm collection, nine experimental hybrids of *C. baccatum* var. *pendulum*, and six *Capsicum* cultivars (Table 1). These genotypes belong to four species of *Capsicum*, being 12 belonging to *C. annuum* var. *annuum*, one of *C. annuum* var. *glabriusculum*, nine of *C. chinense*, eight of *C. frutescens*, and 22 genotypes of *C. baccatum* var. *pendulum*.

When assessing the resistance to BS, accession UENF 1381 (Costa et al., 2002; Riva-Souza et al., 2007) was used as a resistance standard, and Jalapeño M as a susceptible cultivar (control) (Pimenta et al., 2016). For resistance to anthracnose, we only used the susceptible cultivar, 'Ikeda', as a control since there are no *C. gloeosporioides* resistant genotypes (Diao et al., 2017). For resistance to PepYMV, Criollo de Morelos 334 (CM-334) was used as a resistance standard (Nogueira et al., 2012), and 'Ikeda' as a susceptible standard.

Crop conditions

Two experiments were carried out. One of them evaluated the resistance to BS and

to anthracnose (EXP1.1 and EXP1.2). Another evaluation was performed for resistance to PepYMV (EXP2). EXP1.1 and EXP1.2 was conducted in a greenhouse at Centro Estadual de Pesquisa em Agroenergia e Aproveitamento de Resíduos. EXP2 was carried out in cages coated with anti-aphid cloth bags, which were kept in a greenhouse at the Research Support Unit of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). Both experimental fields are located in the city of Campos dos Goytacazes, RJ, Brazil.

Table 1. Species and origin corresponding to the 54 genotypes used in the evaluation of resistance to multiple diseases in *Capsicum* spp. Campos dos Goytacazes, 2016.

Genotypes	Species	Origem	Genotypes	Species	Origem
Criollo de Morellos ¹	<i>C. annuum</i>	IAC	UENF 1775 ¹	<i>C. frutescens</i>	Bequimão, MA
Ikeda ²	<i>C. annuum</i> var. <i>annuum</i>	Porto Alegre	UENF 1776 ¹	<i>C. frutescens</i>	Rosário, MA
UENF 1381 ¹	<i>C. annuum</i> var. <i>annuum</i>	PESAGRO	UENF 1779 ¹	<i>C. frutescens</i>	Bequimão, MA
UENF 1622 ¹	<i>C. annuum</i> var. <i>annuum</i>	Estados Unidos	UENF 1790 ¹	<i>C. frutescens</i>	São Luiz, MA
UENF 1623 ¹	<i>C. annuum</i> var. <i>annuum</i>	Campos, RJ	UENF 1800 ¹	<i>C. frutescens</i>	Bequimão, MA
UENF 1626 ¹	<i>C. annuum</i> var. <i>annuum</i>	Campos, RJ	UENF 1490 ¹	<i>C. baccatum</i> var. <i>pendulum</i>	Coleta - RJ
UENF 1627 ¹	<i>C. annuum</i> var. <i>annuum</i>	Campos, RJ	UENF 1616 ¹	<i>C. baccatum</i> var. <i>pendulum</i>	Viçosa - MG
UENF 1717 ¹	<i>C. annuum</i> var. <i>annuum</i>	Renascença, PR	UENF 1624 ¹	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1740 ¹	<i>C. annuum</i> var. <i>annuum</i>	Cachoeiro de Macacu, RJ	UENF 1629 ¹	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1741 ¹	<i>C. annuum</i> var. <i>annuum</i>	Porto Alegre, RS	UENF 1635 ¹	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF Carioca ³	<i>C. annuum</i> var. <i>annuum</i>	Campos, RJ	UENF 1639 ¹	<i>C. baccatum</i> var. <i>pendulum</i>	Miranda, MS
UENF Camjista ³	<i>C. annuum</i> var. <i>annuum</i>	Campos, RJ	UENF 1714 ¹	<i>C. baccatum</i> var. <i>pendulum</i>	Manhuaçu, MG
UENF Cariquinha ³	<i>C. annuum</i> var. <i>annuum</i>	Campos, RJ	UENF 1718 ¹	<i>C. baccatum</i> var. <i>pendulum</i>	Peru
Jalapeño M ²	<i>C. annuum</i> var. <i>annuum</i>	São Paulo, SP	UENF 1732 ²	<i>C. baccatum</i> var. <i>pendulum</i>	Renascença, PR
UENF 1750 ¹	<i>C. annuum</i> var. <i>glabrusculum</i>	Campos, RJ	UENF 1733 ¹	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1554 ¹	<i>C. chinense</i>	Goiania, GO	UENF 1737 ¹	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1703 ¹	<i>C. chinense</i>	Viçosa, MG	UENF 1797 ¹	<i>C. baccatum</i> var. <i>pendulum</i>	Cachoeiro de Macacu, RJ
UENF 1706 ¹	<i>C. chinense</i>	Viçosa, MG		<i>C. baccatum</i> var. <i>pendulum</i>	Viçosa, MG
UENF 1751 ¹	<i>C. chinense</i>	Parintins, AM	H ³	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1764 ¹	<i>C. chinense</i>	Belém, PA	H ³	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1770 ¹	<i>C. chinense</i>	Belém, PA	H ³	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1772 ¹	<i>C. chinense</i>	Bequimão, MA	H ³	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1780 ¹	<i>C. chinense</i>	Bequimão, MA	H ³	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1798 ¹	<i>C. chinense</i>	Campos, RJ	H ³	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1731 ¹	<i>C. frutescens</i>	Petrolina, PE	H ³	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1747 ¹	<i>C. frutescens</i>	Marajó-Soure, PA	H ³	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ
UENF 1766 ¹	<i>C. frutescens</i>	Belém, PA	H ³	<i>C. baccatum</i> var. <i>pendulum</i>	Campos, RJ

¹Accessions belonging to the BAG/*Capsicum*/UENF; ²commercial witnesses; ³new cultivars developed by UENF; ⁴inbred lines, and ⁵ experimental hybrid.

In both experiments, seeds were sown on 128-cell polystyrene trays, using a commercial substrate (Vivato[®]), remaining in a growing chamber kept at 28°C and a 12-h photoperiod. After reaching four to six true leaves, seedlings were transplanted individually to 5-L pots (EXP1) and 500-mL plastic cups (EXP2), containing a mixture of soil and commercial substrate at a 2:1 ratio.

EXP1.1 - Resistance assessment to BS

The genotypes were distributed in a completely randomized design (CRD), with three replicates, to evaluate BS resistance. For this, two concentrations of bacterial suspension were used. The first contained 1.0×10^8 CFU/mL, being in a hypersensitivity reaction (HR); the second, with 1.0×10^5 CFU/mL, was used in a quantitative resistance evaluation. As inoculum, we used an ENA 4135 isolate, which was previously characterized as *X. euvesicatoria* by Riva-Souza et al. (2007), as classification proposed by Jones et al. (2004) and reported by Potnis et al. (2015). Both concentrations were inoculated on the same day in two distinct leaves of the same plant, duly identified.

The isolate was preserved in water stocks and recovered by transferring the bacterial suspension to Petri dishes containing DYGS medium (Rodrigues Neto et al., 1986), with a platinum loop. After incubation for 36 h at 28°C, bacterial colonies were suspended in

distilled and autoclaved water, and concentration was adjusted to 1.0×10^8 CFU/mL using a spectrophotometer ($A_{600} = 0.3$) (Jones et al., 2000). Starting from this concentration, a serial dilution was performed to reach a concentration of 1.0×10^5 CFU/mL.

Plants were inoculated 15 days after being transplanted by infiltration of bacterial suspension onto the leaf abaxial surface throughout an area of nearly 1.0 cm^2 , using a syringe and hypodermic needles (Riva-Souza et al., 2007; Pimenta et al., 2016).

HR was assessed 24 and 48 h after inoculation; evaluations consisted of presence (1) and absence (0) of local necrosis on the inoculated leaves. For quantitative resistance, evaluations started when the first symptoms were detected, in this case, on the fifth day after inoculation, lasting for nine days and occurring daily at the same time. To do so, scores from 1 to 5 were given according to the scoring range proposed by Riva-Souza et al. (2009). Scores from 1 to 2 were assigned as resistant genotypes, and from 3 to 5 as susceptible, so that the higher the score, the greater the susceptibility to the disease. Afterward, we estimated the area under the disease progress curve (AUDPC), as defined by Campbell and Madden (1990), and the incubation period (IP), which consists of the elapsed days between inoculation and the appearance of the first symptoms. After resistance evaluation, all the plants were grown until reproductive stage for further inoculation of *C. gloeosporioides* on immature and mature fruit.

EXP1.2 - Resistance assessment to anthracnose

For anthracnose, isolate from *C. gloeosporioides* was used, being previously tested, and identified as isolate #8.1. It was inoculated into three fully developed fruits, detached from the same plant (replicates), at both immature (IF) and mature (MF) stages, totaling 972 evaluated fruits.

The isolate was cultured on PDA medium (potato-dextrose-agar) and then incubated in a BOD at 28°C , under a 12-h photoperiod, for seven days. A conidial suspension was prepared in deionized water, sterilized, and adjusted to a concentration of about 1.0×10^6 conidia/ mL in a Neubauer chamber, using an optical microscope.

Fruits were removed from the plants and transferred to the laboratory, being properly identified by genotype. After, they were sanitized by dipping them in a 70% alcohol solution for 1 min and then in a sodium hypochlorite solution (0.2%) for 5 min, after stalk removal. Later, they were three-time washed in sterile deionized water and dried with paper towel.

A conidial suspension (10 μL) was dropped on each fruit; then, we used an entomological needle to wound the dropped area. After inoculation, the fruits were placed in a humid chamber at room temperature ($\pm 28^\circ\text{C}$). Evaluations were performed daily, at the same time, for seven days. Again, we made use of a scoring range, which was suggested by Montri et al. (2009) and used by Silva et al. (2014), thus determining the resistance levels on the seventh day of evaluation. Also, we calculated both incubation (IP) and latent (LP) periods of anthracnose infection, which correspond to the number of days between inoculation and the onset of symptoms, and between inoculation and the appearance of signs (acervulus formation), respectively.

EXP2 - Resistance assessment to PepYMV

In order to evaluate resistance to PepYMV, all 54 genotypes were distributed in a completely randomized design (DIC) with five replications, inside cages coated with anti-aphid cloth bags.

Plants of *Nicotiana debneyi*, infected with PepYMV isolate 3, were used as inoculum source (Truta et al., 2004). Professor Dr. Murilo Zerbini from the Laboratory of Plant Virology, Federal University of Viçosa, kindly provided both *N. debneyi* seeds and the viral isolate.

Inoculation was performed via plant extract buffered in 0.05 M potassium phosphate, pH 7.2, containing 0.01% sodium sulfate, and using 600-mesh carborundum as abrasive (Truta et al., 2004). Plants were inoculated at the stage of 3 to 4 well-developed leaves, being reinoculated after 48 h to avoid escape. For all inoculations, we used young and fully expanded leaves.

Evaluations were made every two days from the 15th day after the first inoculation, which was when the symptoms began to appear. In this case, severity was assessed by score scale tested and validated by Bento et al. (2009), wherein: 1- absence of symptoms; 2- slight symptoms (25% leaf area with small mosaic areas); 3 - medium symptoms (50% leaf area with mosaic); 4 - intense symptoms (75% leaf area with mosaic), and 5 - severe symptoms (100% leaf area with mosaic, blisters, leaf twisting and area reduction). After phenotyping, AUDPC and IP were estimated.

Statistics

The Lilliefors test was used to determine data normality. After, an individual analysis of variance (ANOVA) was performed. The means were grouped by the Scott-Knott test ($P < 0.05$), using the Genes software (Cruz, 2013).

For anthracnose, in particular, symptoms observed on the last day (seventh day) were classified according to Montri et al. (2009). In accordance with mean scores of symptom observed in each accession, we could rate them according to their level of resistance.

To avoid unsure resistance findings, based on a single variable, data underwent multivariate analysis for all tested variables, and for both BS and PepYMV. Thus, we made use of the algorithm of Gower (1971), thus obtaining the dissimilarity matrix among the genotypes. A simplified representation of genetic distances between genotypes was achieved by unweighted pair group method using arithmetic average (UPGMA) hierarchical grouping method. Matrices, distances, and the UPGMA grouping were performed using the R software package (<http://www.r-project.org>).

RESULTS

EXP1.1 - Resistance assessment to BS

The studied genotypes showed variability for resistance to BS. Seventeen of them were resistant according to the HR test (14 accessions of *C. baccatum* var. *pendulum*, two of *C. frutescens*, and one of *C. annuum* var. *annuum*), being hypersensitive to the pathogen.

We inoculated in the evaluated genotypes only one isolate of *X. euvesicatoria* (previously strain T1P3); however, a relatively high number of genotypes reacted (32%).

The AUDPC for BS ranged between 8.0 and 35.8, averaging 23.43. Twenty genotypes were considered resistant to this disease, since the standard accession, UENF 1381, was grouped to another 19 genotypes by the Scott-Knott test (Table 2). Among them, eleven are *C. baccatum* var. *pendulum*, five are *C. annuum* var. *annuum*, one is *C. annuum* var. *glabriusculum*, and two are *C. chinense*. No accessions were solely resistant to *C. frutescens* when considering the AUDPC.

Table 2. Clustering of averages by Scott Knott ($P < 0.05$) of 54 genotypes of *Capsicum* spp relative to the variables (AUDPC), hypersensitivity reaction (HR) and incubation period (IP) of the bacterial spot and the yellow pepper mosaic. Campos dos Goytacazes, 2016.

Genotypes	Bacterial			Virus		Genotypes	Bacterial			Virus	
	AUDPC ¹ Average	IP Average	HR	AUDPC Average	IP Average		AUDPC Average	IP Average	HR	AUDPC Average	IP Average
UENF 1381	08.00 b	15.00 a	0	25.20 c	27.40 b	UENF 1766	27.00 a	08.00 b	0	25.80 c	21.00 c
UENF 1490	08.50 b	14.67 a	1	14.50 d	34.20 a	UENF 1766	29.16 a	08.67 b	0	44.70 b	11.40 d
UENF 1554	23.83 a	07.67 b	0	24.40 c	25.80 b	UENF 1770	17.60 b	10.30 b	0	14.00 d	35.00 a
UENF 1616	28.00 a	07.32 b	1	14.00 d	35.00 a	UENF 1772	34.60 a	07.67 b	0	NR	NR
UENF 1622	21.50 a	10.00 b	0	NR	NR	UENF 1775	34.50 a	07.33 b	0	47.40 a	16.20 c
UENF 1623	22.60 a	10.00 b	1	52.00 a	11.00 d	UENF 1776	34.80 a	07.33 b	0	44.60 b	14.20 d
UENF 1624	08.00 b	15.00 a	1	14.00 d	35.00 a	UENF 1779	35.80 a	07.33 b	1	54.00 a	11.00 d
UENF 1626	16.60 b	12.33 a	0	56.00 a	11.00 d	UENF 1780	32.50 a	07.67 b	0	37.40 b	21.00 c
UENF 1627	22.00 a	09.00 b	0	57.40 a	11.00 d	UENF 1790	32.83 a	08.00 b	0	48.90 a	11.40 d
UENF 1628	26.30 a	10.00 b	0	46.80 a	11.00 d	UENF 1797	27.50 a	07.67 b	1	34.40 b	13.80 d
UENF 1629	08.30 b	15.00 a	0	47.04 a	11.00 d	UENF 1798	28.00 a	08.00 b	0	26.50 c	11.00 d
UENF 1635	17.50 b	12.33 a	0	57.20 a	11.00 d	UENF 1800	34.60 a	07.33 b	0	54.00 a	11.00 d
UENF 1639	09.50 b	13.67 a	0	48.00 a	11.00 d	H2	23.60 a	07.33 b	0	26.70 c	15.40 c
UENF 1703	12.00 b	11.30 a	0	14.00 d	35.00 a	H3	32.16 a	07.00 b	1	37.30 b	11.00 d
UENF 1706	34.80 a	07.67 b	0	14.00 d	35.00 a	H4	20.46 b	10.00 b	1	14.00 d	35.00 a
UENF 1714	30.10 a	08.33 b	1	14.00 d	35.00 a	H5	19.00 b	07.33 b	1	22.30 c	20.20 c
UENF 1717	33.60 a	07.33 b	0	54.20 a	11.00 d	H6	30.60 a	09.50 b	1	15.70 d	31.80 a
UENF 1718	15.10 b	10.30 b	1	44.00 b	11.80 d	H7	14.75 b	11.67 a	0	14.50 d	34.33 a
UENF 1731	34.16 a	07.33 b	1	45.30 b	11.80 d	H8	14.16 b	11.00 a	0	41.70 a	11.00 d
UENF 1732	30.60 a	07.33 b	0	15.10 d	33.80 a	H9	15.16 b	07.33 b	1	27.20 c	18.20 c
UENF 1733	35.00 a	07.33 b	1	14.00 d	35.00 a	H10	28.60 a	09.00 b	1	36.30 b	11.40 d
UENF 1737	33.10 a	07.33 b	1	14.00 d	35.00 a	"Criolo de Morelos"	20.50 b	09.67 b	0	14.00 d	35.00 a
UENF 1740	23.50 a	08.00 b	0	47.80 a	16.20 c	"UENF Campista"	08.00 b	15.00 b	0	27.00 c	21.80 c
UENF 1741	32.30 a	07.33 b	0	53.40 a	11.00 d	"UENF Carioquinha"	08.00 b	15.00 b	0	37.40 b	16.20 c
UENF 1747	35.16 a	07.33 b	0	49.60 a	11.80 d	"UENF Carroca"	08.00 b	15.00 b	0	30.60 c	21.80 c
UENF 1750	08.00 b	15.00 a	0	29.40 c	25.40 b	"Jalapeño M"	24.33 a	07.00 b	0	19.40 d	31.00 a
UENF 1751	30.30 a	08.00 b	0	14.00 d	35.00 a	"IKEDA"	NR	NR	NR	43.00 b	14.20 d

¹AUDPC = area under the disease progress; IP = incubation period; NR = not rated.

BS IP varied from seven to 15 days, with an average of 10 days. Fourteen genotypes were within the same group with the highest IP values, i.e., at or above 11 days. These genotypes include all species studied here, except for *C. frutescens*, likewise the results observed for AUDPC (Table 2). The experimental hybrids H6 and H7 were resistant to BS and, despite not having any relationship (same parent), they have agronomic characteristics required by consumer market (Gonçalves et al., 2011; Bento et al., 2016) and were deemed promising to be released to farmers after cropping values tests. Genotype UENF 1750 (*C. annuum* var. *glabriusculum*) had the lowest value for AUDPC (8.00) and higher value for IP (15).

EXP1.2 - Resistance assessment to anthracnose

Only accession UENF 1381 was classified as resistant to anthracnose, for immature fruit, based on the scale of Montri et al. (2009), on the 17th day. The others were susceptible at different levels, among which three were moderately susceptible, four susceptible and 46 highly susceptible. For mature fruit, there were no resistance genotypes; however, 16 of them were moderately resistant, including UENF 1381. It was required an IP of three days for immature fruit, and four days for mature ones. Regarding LP, immature fruits needed six days and eight days for mature ones. Accession UENF 1381 had the highest value for IP and LP for immature fruit, without any symptom, considering eight days. For mature fruit, this accession showed a five-day IP and an eight-day LP, that is, presented symptoms but did not produce any signs.

EXP2 - Resistance assessment to PepYMV

Regarding PepYMV, AUDPC and IP for the virus ranged from 14 to 57.4 and from 11 to 35 days, respectively. Considering both of them, we verified resistance in 16 genotypes, four of which were for *C. chinense*, ten for *C. baccatum* var. *pendulum*, and two for *C. annuum* var.

annuum (Table 2). The hybrids of *C. baccatum*, H5, and H6, were considered promising because they obtained two desirable resistance components to PepYMV, which were AUDPC and IP.

Multivariate analysis

Based on the multivariate analysis, six groups were identified in the UPGMA dendrogram at a dissimilarity level of 0.30, with distinct resistance traits. The first group was characterized as being intermediate for resistance to BS, with no HR and being highly resistant to PepYMV. This group consisted of four accessions, including the virus resistance standard - ‘Criollo de Morelos’ (Figure 1).

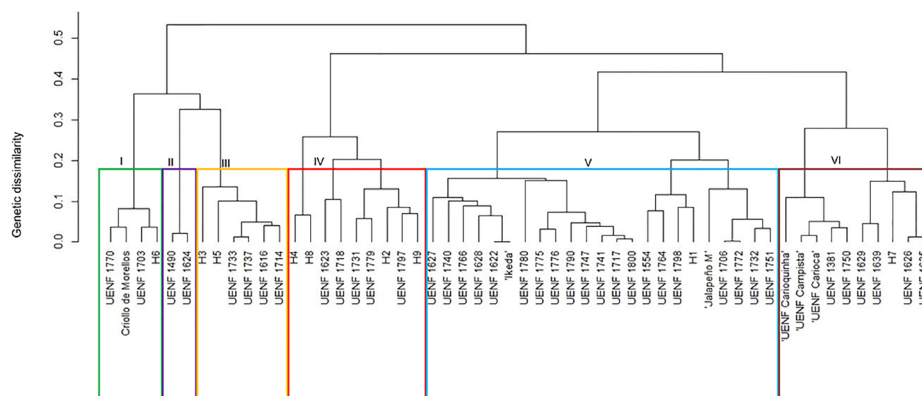


Figure 1. Dendrogram obtained by the UPGMA method for the variables below area under the disease progress curve (AUDPC), hypersensitivity reaction (HR) and incubation period (IP) of bacterial spot and yellow pepper mosaic. Campos dos Goytacazes, 2016.

The second group was highly resistant to both BS and to the virus, showing highly favorable results for all evaluated resistance variables. This group consisted of two accessions of *C. baccatum* var. *pendulum* (UENF 1490 and UENF 1624). Group III was formed only by genotypes of *C. baccatum* var. *pendulum*, which was susceptible to quantitative resistance to BS; however, all of them showed hypersensitivity reaction to the bacterium and were highly resistant to PepYMV.

The fourth and fifth groups included nine and 23 genotypes, respectively. These groups were susceptible to BS, for quantitative resistance, and to PepYMV. However, they differed regarding HR, and only the fourth group showed genotypes with such resistance. The sixth and last group consisted of ten genotypes resembling each other, once they had only quantitative resistance to BS, that is, without HR expression and being moderately susceptible to PepYMV. Accession UENF 1381 was allocated to this last group, which is the resistance standard to BS, besides three cultivars derived from this accession.

DISCUSSION

EXP1.1 - Resistance assessment to BS

The variability found for the hypersensitivity reaction is important and may contribute

to the breeding of new commercial genotypes of both sweet and chili peppers. Plant hypersensitivity to a particular pathogen can narrow and eventually kill it, thus contributing to the disease control (Kombrink and Schmelzer, 2001; Wai et al., 2015).

However, HR is considered provisional since the longevity of a BS resistance gene depends on the stability of pathogen's *avr* gene. Nevertheless, combining several HR genes into the same cultivar can confer a prolonged resistance (Stall et al., 2009).

Following the gene-to-gene hypothesis proposed by Flor (1955), three non-allelic and dominant genes (*Bs1*, *Bs2*, and *Bs3*) might have conferred resistance to BS, being expressed phenotypically by means of HR (Hibberd et al., 1987). Nonetheless, other interactive genes with avirulence alleles (*avrBsT*, *avrBs4C* and *avrBs7*) have been described in bacterial isolates, promoting HR. As examples, we may cite *BsT* (Minsavage et al., 1990), *Bs4C* (Straub et al., 2012), and *Bs7* (Potnis et al., 2012).

One-third of the genotypes showed a hypersensitivity reaction to a single isolated *X. euvesicatoria* (T1P3). This result leads us to assume that more than one allele might be responsible for these phenotypes; and, for race 3, two resistance genes (*Bs2* and *Bs4*) are associated to resistance in the same plant. Thus, it is feasible to use multilines for these genotypes, which serves as a strategy to increase resistance stability (Stall et al., 2009).

By the results obtained for the AUDPC-BS six genotypes of *C. annuum* were resistant, one is the resistance pattern (UENF 1381), as already expected, three genotypes ('UENF Campista', 'UENF Carioquinha', and 'UENF Carioca') were resistant since they are cultivars developed from crosses involving UENF 1381 (Riva-Souza et al., 2007, 2009; Pimenta et al., 2016). Three recessive genes confer genetic control of BS resistance (Riva et al., 2004b); therefore, the studied cultivars are homozygous for this trait.

Genotype UENF 1750 (*C. annuum* var. *glabriusculum*) had relatively low values for AUDPC and high for IP. Such observation is important because this genotype is little exploited commercially and can be used in breeding programs for new cultivars; since to date, there is no record in Brazil of any commercial cultivar of this species (Brasil, 2015). In Mexico, consumers who pay high prices considered one variety of *C. annuum* var. *glabriusculum* as 'premium'. This preference is mainly due to its better flavor whether compared to the other types of peppers such as Serrano and Jalapeño (Villalon-Mendoza et al., 2014).

EXP1.2 - Resistance assessment to anthracnose

In relation to anthracnose, only one genotype (UENF 1381) in immature fruit was resistant and the others were classified as susceptible in different degrees of aggressiveness, and in the mature fruit this genotype was moderately resistant as well as a further 15 genotypes and the rest susceptible. These results demonstrate the ongoing aggressiveness of isolate #8.1, as well as UENF 1381 accession resistance.

The genetic control of anthracnose resistance is considered complex because it varies according to *Colletotrichum* species, isolate, and host species (Ying et al., 2015). In *Capsicum* genus, resistance control depends on the plant organ under study, such as leaf or fruit (Mahasuk et al., 2009a). In the case of fruit, resistance may also vary with maturation stage, whether immature or mature (Silva et al., 2014; Sun et al., 2015; Suwor et al., 2015). In this study, we noticed that immature fruit was more susceptible to anthracnose than mature ones, which corroborates the hypothesis of different genetic control in these maturation stages.

The Asian Vegetable Research Development Center (AVRDC) identified, in 1997 and

in 1999, the main sources of anthracnose resistance in *Capsicum* plants for *C. baccatum* and *C. chinense* (Pakdeevaporn et al., 2005). Since then, several researchers have used these genotypes in *Capsicum* breeding programs for resistance to anthracnose. As examples, we may cite studies developed by Pakdeevaporn et al. (2005) and Mahasuk et al. (2009a), who crossed PBC932 (*C. chinense*) with 'Bangchang' (*C. annuum*). These authors noted that resistance was controlled by one gene (co1) in green fruit, by another (co2) red fruit, and yet another (co3) in seedlings, when using a *C. capsici* isolate identified as '158ci'. In addition, Mahasuk et al. (2009b) evaluated *C. baccatum* progenies from PBC80 x PBC1422 crossing, comprising 13 isolates of *C. capsici* and *C. acutatum*, found two new genes: *co4* (controlling resistance in green fruit) and *Co5* (controlling resistance in red fruit). The genetic control of anthracnose resistance for accession UENF 1381 has not yet been identified, therefore, determining it may contribute to the development of new resistant cultivars and to the better understanding of plant-pathogen interaction.

EXP2 - Resistance assessment to PepYMV

PepYMV resistance sources in some species of *Capsicum* have already been reported, such as for *C. annuum*, cultivars 'Perennial' and 'Magali R' (Truta et al., 2004; Nascimento et al., 2007), two accessions of *C. baccatum* var. *pendulum*, seven species of *C. chinense* (Bento et al., 2009), and cultivar 'BRS Juruti' (*C. chinense*) (Ribeiro et al., 2015), among others. However, few papers have cited multiple resistance including PepYMV, one of the few examples is the study of Nascimento et al. (2007). In this research, these authors evaluated *C. annuum* strains, cultivars, and hybrids for resistance to *Phytophthora capsici* and to PepYMV; they found resistance to both pathogens in two progenies, and one hybrid of *C. annuum*.

Multivariate analysis

By the UPGMA method the first group was highlighted as being highly resistant to PepYMV, including the resistance pattern. In this group the genotype UENF 1703 of *C. chinense* showed no hypersensitivity reaction, it was moderately resistant to bacterial spot, and could be considered a promising genotype for resistance source to develop new cultivars from *C. chinense* that are resistant to both BS and to PepYMV.

The two accessions of second group have already been characterized morpho-agronomically and molecularly (Leite et al., 2016) and showed traits of interest for the pepper market such as productivity, intense red fruit color when mature, total soluble solids content near to 7.0° Brix, among others (Bento et al., 2009; Assis, 2014; Leite et al., 2016). Assis (2014) assessed 30 genotypes of *C. baccatum*, including these two accessions, pointing out 100% antioxidant activity to UENF 1490. Therefore, when we admit that promising accessions have not yet been studied, from a breeding standpoint, a mass selection can be applied to provide resistant strains with good agronomic characteristics. In addition, these accessions may also be recommended as parents of breeding programs, for resistance to both diseases.

CONCLUSIONS

Capsicum frutescens was most susceptible to the three diseases jointly. At least one genotype of *C. baccatum*, *C. annuum*, and *C. chinense* had a potential for use in breeding

programs for resistance to bacterial spot and PepYMV. The genotypes UENF 1490 and UENF 1624, both of *C. baccatum* var. *pendulum*, were highly resistant regarding all resistance variables to bacterial spot and PepYMV.

The genotype UENF 1381 (*C. annuum*) was the only one presenting multiple resistance to the three pathogens, being highly resistant to bacterial spot and anthracnose in immature fruit, and moderately resistant to PepYMV and anthracnose in mature fruit. UENF 1381 is a promising genotype to be further used in breeding programs to disease resistance in *Capsicum*.

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