GENETIC RESISTANCE TO *Xanthomonas euvesicatoria* AND *X. gardneri* IN *Capsicum annuum*: INHERITANCE STUDIES AND THE PROPOSAL OF A NEW GENE INVOLVED IN THE HYPERSENSITIVE RESPONSE

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Aprovada em 26 de fevereiro de 2018.

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To GOD, to my beloved mother, Ivane, my sisters, Lídia Raquel and Maria Carolina, my nephews, Ana Sofia and Benjamim.

I offer.

To the loves that God has placed in my life, Joviana Lerin and Vivian Melo, for supporting me and holding me daily, and Luis Jonathan for friendship and love.

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SUMMARY

RESUMO

SILVA, L. R. A. da. D.Sc; Universidade Estadual do Norte Fluminense Darcy Ribeiro; Fevereiro de 2018. Resistência genética a *Xanthomonas euvesicatoria* e *X. gardneri* em *Capsicum annuum*: estudos de herança e proposta de um novo gene para Reação Hipersensível. Orientadora: Profa. Rosana Rodrigues; Conselheiros: Prof. Alexandre Pio Viana e Profa. Helaine Christine Cancela Ramos.

A mancha bacteriana, causada por *Xanthomonas* spp.*,* é uma das doenças mais destrutivas para a cultura do pimentão. O uso de cultivares resistentes é a forma mais apropriada de controle, e para o desenvolvimento dessas cultivares o conhecimento da genética que controla a resistência é primordial, pois orienta a escolha do método de melhoramento mais apropriado. Os objetivos deste trabalho foram: estudar a herança genética da resistência à mancha bacteriana em uma população oriunda do cruzamento entre genótipo suscetível e resistente de *Capsicum annuum*; selecionar plantas da geração F_2 desse cruzamento resistentes à mancha bacteriana; verificar se a resistência presente no acesso UENF 1381 é condicionada pelos genes *bs5* e *bs6*, já descritos na literatura; e testar o alcance da resistência encontrada no acesso UENF 1381 em relação a diferentes espécies e raças de *Xanthomonas*. Na primeira etapa, os parentais P_1 (UENF 2285, suscetível), P_2 (UENF 1381, resistente), as gerações F_1, F_2, RC_1 e RC_2 foram conduzidos em casa de vegetação, na Unidade de Apoio à Pesquisa da UENF. As plantas de cada geração foram inoculadas com o isolado ENA 4135 na concentração de 10^5 ufc mL^{-1} em 1 cm^2 do mesófilo. Para avaliação utilizou-se

uma escala descritiva de notas variando de um (resistente) a cinco (suscetível), dependendo no nível de manifestação dos sintomas. Pela análise quantitativa foi estimado um número mínimo de cinco genes que controlam a resistência à mancha bacteriana. O efeito aditivo (6,06) foi superior ao dominante (3,31) e explicou 86,36% da variação total. Com o valor do grau médio de dominância obtido demonstrou tratar-se de uma dominância parcial. Uma análise das gerações utilizando o método da máxima verossimilhança foi empregada para identificar o efeito dos genes, e se existe ou não influência de genes de dominância na expressão da característica. Os resultados dessa análise mostraram que a resistência à mancha bacteriana é poligênica, com pelo menos um gene de efeito maior com efeito aditivo, associado a poligenes com efeitos aditivos e de dominância, sendo uma característica de natureza genética complexa. A seleção de genótipos resistentes à mancha bacteriana e com formato de pimentão foi efetuada utilizando-se o índice de Mulamba e Mock. Pode-se assim ranquear genótipos com as características de interesse associadas com outras de qualidades nutricionais do fruto. Para verificar a presença dos genes *Bs4*, *bs5* e *bs6* uma análise molecular baseada em reação de polimerase em cadeia foi conduzida em parceria com a *University of Florida* (UF) utilizando-se iniciadores específicos para detecção dos genes em questão. Também na UF, foi feita a determinação do alcance da resistência no acesso UENF 1381, e da geração F_2 do cruzamento em estudo, inoculando-se diferentes espécies de *Xanthomonas* em condições controladas. Reação de hipersensibilidade (RH) foi identificada tanto no UENF 1381 como em alguns genótipos da geração F_2. Como não foi identificada a presença dos genes *Bs4*, *bs5* e *bs6* é possível propor a existência de um novo gene responsável pela RH em *Capsicum annuum*. Com os resultados deste trabalho será possível traçar um programa de melhoramento a partir deste cruzamento, que vise o desenvolvimento de linhagens de pimentão com resistência à mancha bacteriana e com características agronômicas desejáveis.

ABSTRACT

SILVA, L.R.A. da. D.Sc; Universidade Estadual do Norte Fluminense Darcy Ribeiro; February 2018. Genetic resistance to *Xanthomonas euvesicatoria* and *X. gardneri* in *Capsicum annuum*: inheritance studies and proposal of a new gene for Hypersensitive Reaction. Advisor: Profa. Rosana Rodrigues; Commitee members: Professor Alexandre Pio Viana and Professor Helaine Christine Cancela Ramos.

The bacterial spot, caused by *Xanthomonas* spp., is one of the most destructive diseases for the pepper crop. The use of resistant cultivars is the most appropriate form of control and for the development of these cultivars it is necessary to know the genetic base that controls the resistance, allowing the selection of the most appropriate breeding method. The objectives of this work were to study the genetic inheritance of resistance to bacterial spot in a population from the cross between susceptible and resistant genotype of *Capsicum annuum*; to select plants of the F_2 generation of this crossing resistant to the bacterial spot to give continuity to the breeding program; to verify if the resistance present in UENF 1381 is conditioned by genes *bs5* and *bs6*, already described in the literature; and to test the extent of resistance found in UENF 1381 access to different species and races of *Xanthomonas*. In the first stage, the generations P_1 (UENF 2285, susceptible), P_2 (UENF 1381, resistant), F_1, F_2, RC_1 and RC_2 were conducted under greenhouse conditions at the UENF. The plants of each generation were inoculated with the isolate ENA 4135 at the concentration of 10^5 cfu mL^{-1} in 1 cm^2 of the mesophyll. A

descriptive scale of scores ranging from one (resistant) to five (susceptible) was used for evaluation, depending on the level of manifestation of the symptoms. By the quantitative analysis, a minimum number of five genes that control resistance to bacterial blight in the study populations were estimated. The additive effect (6.06) was higher than the dominant (3.31) and explained 86.36% of the total variation. With the value of the average degree of dominance obtained it was shown to be a partial dominance. An analysis of the generations using the maximum likelihood method was used to identify this effect of the gene and to explain the results of the first experiment. Resistance to bacterial blight is polygenic, with at least one gene having a greater effect with additive effect, associated with polygenes with additive and dominance effects, being a characteristic of a complex genetic nature. Selection of genotypes resistant to bacterial and pepper blot was performed using the Mulamba and Mock index, with which it was possible to rank genotypes with the characteristics of interest associated with others of nutritional qualities of the fruit. To verify the presence of *Bs4*, *bs5* and *bs6* genes a molecular analysis based on polymerase chain reaction was conducted in partnership with the University of Florida using specific primers to detect the genes in question. Finally, to determine the extent of the resistance of UENF 1381 and of the F_2 generation of the cross under study, inoculations with different species of *Xanthomonas* were carried out under controlled conditions. Hypersensitivity reactions (HR) were identified in both the UENF 1381 and some genotypes of the F_2 generation. As the presence of *Bs4*, *bs5* and *bs6* genes was not identified, it is possible to propose the existence of a new gene responsible for HR in *Capsicum annuum*. With the results of this work it will be possible to draw a breeding program from this crossroads, aiming the development of bacterial lines resistant to bacterial blight and with desirable agronomic characteristics.

1. INTRODUCTION

Sweet and chili peppers (*Capsicum* spp.) are vegetables widely appreciated worldwide due to their wide variability in color, shape and fruit flavor, besides generating raw material for the food, pharmaceutical and cosmetic industries as well as the market for ornamental plants (Rêgo et al., 2011). The cultivation of the plants of this genus in Brazil is present in the different regions due to the low cost of production per hectare when compared to other vegetables (Costa and Henz, 2007).

A limiting factor to plant cultivation of this genus is the high sensitivity to pests and diseases, which reduces yield and fruit quality (Rêgo et al., 2011). One of the most relevant diseases is the bacterial spot (*Xanthomonas* spp.), which causes defoliation of the plants and consequently compromises the production of fruits ideal for commercialization (Potnis et al., 2015).

Disease control becomes more effective, considering economic and ecological aspects, when several strategies are used in an integrated way. Rezende et al. (2005) found that the use of genetic resistance represents one of the most efficient, easily accessible and inexpensive methods of control, reducing disease losses and production costs significantly.

Since 2008, Brazil has been among the largest consumers of chemical pesticides. In the specific case of sweet pepper, the report of the *Associação Brasileira de Saúde Coletiva* (ABRASCO) showed the sweet pepper as the crop with the highest contamination index, counting 91.8% of the samples with

pesticide residues, followed by strawberry (63.4%) and cucumber (57.4%) (IBGE, 2009; ABRASCO, 2015).

Genetic resistance to diseases is an effective mechanism, which aims to minimize contamination of the environment and food by the continuous use of pesticides (Riva-Souza et al., 2009). The genetic control of resistance to bacterial blight in peppers, with the identification of resistance sources and the search for resistant cultivars has been the focus of studies both abroad and in Brazil (Cook e Stall, 1982; Riva et al., 2004; Riva-Souza et al., 2007; Moreira et al., 2015; Silva et al., 2017).

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In addition to estimating the number of genes, it is crucial to know the type of inheritance that controls the resistance of a particular disease. The genetic study of generations quantifies the nature and the available genetic variability, besides evaluating the importance of the genetic effects that constitute the averages of the populations studied (Cruz et al., 2014). Knowledge of the type of interaction involved in a population is important to make an appropriate decision in plant breeding (Malhotra and Singh, 1989).

In order to perform the selection of plants that have a set of characteristics that results in obtaining a genotype that surpasses what exists in the market, there are strategies used in the improvement that help this combination. The selection index proposed by Mulamba and Mock (1978) is one of those techniques used by breeders, since it classifies the genotypes according to the importance of the characteristics of interests.

The search for new resistant genotypes is essential, because there is a need for products that require less agricultural pesticides. In the Brazilian market, there are only hybrids of peppers resistant to bacterial blight. Recently, the Program of Genetics and Plant Breeding of the Universidade Estadual do Norte Fluminense (UENF) has launched three cultivars, pure lines of pepper resistant to bacterial blight (Pimenta et al., 2016). The researches of this program advance to obtain chili cultivars resistant to this disease.

2. OBJECTIVES

2.1. General objective

To study genetic aspects of resistance to bacterial spot in *Capsicum*-*Xanthomonas* interaction.

2.2. Specific objectives

a) Obtaining hybrid F_1 and segregating populations F_2, backcrosses (BC_1 and BC_2) from the biparental cross between UENF 2285 (susceptible) and UENF 1381 (resistant);

b) Phenotyping the population of *Capsicum annuum* for resistance to bacterial spot and eight agronomic characters;

c) Studying the inheritance of resistance to bacterial blight, estimating genetic parameters such as heritability, minimum number of genes, and the genetic effects associated to this character, using different approaches;

d) Selecting superior genotypes for the characters of interest using selection index;

e) Verifying the presence of *Bs4*, *bs5* and *bs6* genes in resistant accession UENF 1381 and in F_2 plants from the study cross; and

f) Analyzing the extent of resistance to bacterial spot in the accession UENF 1381 against different isolates of *Xanthomonas* spp.

3. LITERATURE REVIEW

3.1. General aspects of the genus *Capsicum*

The species of the genus *Capsicum* belongs to the division Spermatophyta, filo Angiospermae, class Dycotiledoneae, order Solanales and family Solanaceae (Andrews, 1995). It is composed of about 35 taxa (species and their varieties) (Bianchetti and Carvalho, 2005), having five domesticated species: *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens*, differentiating between the color, number and floral position, as well as leaf and fruit anatomy (Moscone et al., 2007, Dias et al., 2013). Three new species of *Capsicum* have been described: *C. caatingae*, *C. longidentatum* (Barbosa et al., 2011) and *C. eshbaughii* (Barbosa, 2011).

The species of *Capsicum* are native to the American continent, more precisely from tropical and temperate regions, from Mexico to Brazil (Garcia et al., 2016). The origin was in the mountainous regions of Bolivia (Chiou and Hastorf, 2014) where it later underwent a process of dispersion into the Andes and lowlands of the Amazon (Moscone et al., 2007). One of the forms of this dispersal was through birds migrating to these regions (Stommel and Bosland, 2005). In 1997, Pickersgill proposes the hypothesis that the species *C. annuum* and *C. frutescens* were domesticated in Mesoamerica, *C*. *chinense*, *C*. *baccatum* and *C*. *pubescens*, in South America.

Brazil is a secondary center for the diversity of domesticated species such as C. *annuum* var. *annuum*, *C. baccatum* var. *pendulum*, *C. frutescens* and *C.*

chinense and has greater variability for these species (Reifschneider, 2000). This is due to the presence of wild, semidomesticated and domesticated species (Monteiro et al., 2010; Nascimento et al., 2012). The center of diversity of *C*. *annuum* var. *annuum*, the most variable and cultivated form, includes Mexico and Central America (Embrapa, 2007).

Agronomically, plants of the genus *Capsicum* are identified as olericulture (Oliveira et al., 2014) and the species are divided into three gene complexes that differ according to crossability. The *C*. *annuum* complex comprises the species *C*. *annuum* (varieties *annuum* and *glabriusculum*), *C*. *frutescens*, *C*. *chinense*, *C*. *chacoense* and *C*. *galapagoense* (Zijlstra et al., 1991); the *C. baccatum* complex contains the species *C. baccatum* var. *pendulum* (cultivated form), *C. baccatum* var. *baccatum* (wild type) and *C. tovarii*; and *C*. *pubescens* complexes includes *C. pubescens*, *C. cardenasii* and *C. eximium* (Moscone et al., 2007, Ince et al., 2010; Martins et al., 2010).

The *Capsicum* species present perfect and self-compatible flowers, favoring spontaneous self-fertilization (Bosland, 1996). Studies have shown that cross-pollination in plants of this genus can occur in a range of 0.5 to 70%, characterizing them as mixed pollination species, which can be classified into an intermediate group between allogamous and autogamous, associated with the presence of insect pollinators (Nascimento et al., 2006). When self-fertilization occurs naturally, due to the percentage of cross-fertilization already observed in *Capsicum*, the development of pure inbred lines is compromised. In such cases, artificial self-pollination, aided by measures to prevent contamination of pollen, has been a strategy used (Rêgo et al., 2012).

According to Carvalho and Bianchetti (2008) the plants of C. *annuum* present a flower by knot and in the anthesis the pedicels can be erect, sloping or inclined. The corolla is white (without blemishes) at the base of the lobes of the petals and the anthers are bluish. The calyx of the flower is slightly dentate and has no annular constriction at the junction of the pedicel and are hermaphrodite. Sweet pepper plants of *Capsicum* are diploids, with the number of chromosomes varying between species, being divided into two groups: some with 2n = 2x = 24 and other wild species with 2n = 2x = 26 (Moscone, 2007).

The plant has semi-woody stem, being able to reach 1.0 meters of height. Its fruits are usually hollow, pendent berries with varying shapes, sizes and colors

and pungency (Reifschneider, 2000; Lim, 2013).

3.2. Nutritional and pharmaceutical importance

The fruits of sweet and chili peppers are appreciated all over the world, due to their wide variety of by-products, presenting great economic importance.

The largest world producers of *Capsicum in natura* are China (15 million tonnes/year), followed by Mexico (2.8 million tonnes/year) and Turkey (1.9 million tonnes/year) (FAOSTAT, 2016). The areas cultivated with *Capsicum* plants in these countries are 711.696, 143.465 and 101.000 hectares, respectively (FAO, 2017).

In 2011 the production of pepper, in the largest producing countries, grew 33.3 million tons planted in 3.8 hectares. That same year the value of the global production of pepper was US $ 14.4 billion. This information demonstrates a considerable increase in the last decades in the consumption of pepper. This consumption can be attributed to the high nutritional value of this fruit (Kim et al., 2014).

In Brazil, the major producing states are Minas Gerais, Goiás, São Paulo, Ceará and Rio Grande do Sul (Paulus et al., 2015). The production of peppers in these states in 2011 occupied an area of 2,000 hectares, with yield ranging from 10 to 30 t ha^{-1} (Paula et al., 2011). The growing domestic demand has driven the increase of cultivated area and the establishment of agroindustries, which makes *Capsicum* agribusiness important for Brazil (Panorama Rural, 2006; Rêgo et al., 2011).

Agribusiness involving only *C*. *annuum* exerts importance in family agriculture and in the integration of the small farmer with the agroindustry (Ribeiro and Cruz, 2004). Due to its high capacity to generate employment and income, and its production cost per hectare is relatively low, especially when compared to the costs of other vegetables, this species is positioned within Brazilian agriculture as being of great socioeconomic value (Costa e Henz, 2007).

In Brazil, peppers are consumed *in natura* or in processed form, adding value in the by-products, such as sugar confectionery, chocolates, ethnical spicy foods, among others (Rêgo et al., 2012; Barroca et al., 2015).

Capsicum fruits are also widely used in the pharmaceutical and cosmetic

industries because they are source of vitamins and antioxidant, antimicrobial, anti-cancer, antiarthritic and analgesic properties (Custódio et al., 2010; Moraes et al., 2013). They are sources of vitamins A, B1, B2, C and E (Viñals et al., 1996; Wahyuni et al., 2013). The fruits have also flavonoids including apigenin which has antioxidant capacity and effective action in the treatment of cancerous and neurological diseases (Carvalho et al., 2010; Bae et al., 2014; Zhang et al., 2015). In addition, the genus *Capsicum*, due to its antibacterial properties, has an effect on caries-causing bacteria, *Streptococcus mutans*, with potential for the prevention of cariogenic processes (Carvalho et al., 2010; Santos et al., 2011).

3.3. Disease as a limiting factor for the cultivation of *Capsicum*: the bacterial spot and the control of the disease through genetic resistance

Sweet and chili peppers are the target of several diseases reported in the literature (Marame et al., 2010). Among them, those of fungal etiology, such as anthracnose, phytophthora wilt and powdery mildew, stand out; among the viral diseases, the most important are PVY (Potato virus Y), PepYMV (Pepper yellow mosaic virus), TMV (Tobacco mosaic virus) and tospovirus (Carmo et al., 2006); and regarding bacterial diseases, the bacterial spot (*Xanthomonas* spp.) (Jones et al., 2004) is the major concern of plant breeders and phytopathologists.

The bacterial spot, caused by species of *Xanthomonas*, is considered the main bacterial disease in *Capsicum*. The causal agent is a gram-negative, bacilliform bacterium, movable by means of polar flagella, forming a smooth yellow colony (Jones et al., 2004). It can cause significant leaf damage, both in the protected environment and in the field, leading to loss of fruit production and quality (Riva-Souza et al., 2009; Hamza et al., 2010).

The damages caused by this disease are due to the reduction of productivity by foliar destruction, with consequent loss of photosynthesizing surface and falling of flowers and fruits in formation (Lopes and Quezado-Duval, 2005). It is a disease of difficult control and several factors contribute to this, such as variable efficiency of chemical control, with few products registered for this purpose; unavailability of cultivars with adequate resistance; rapid dissemination in crops under favorable conditions, high relative humidity and contaminated seed transmission (Lopes and Quezado-Soares, 2000).

The infection occurs through natural wounds and openings (stomata and hidatodes) and the colonization of the intercellular spaces is localized, with visible symptoms such as soaked lesions, later necrosis, causing defoliation and severely stained fruits, resulting in great economic losses for the culture. The infectious process is favored by relative humidity between 95 and 100%, temperatures between 22 and 32 °C and occurs at any stage of plant development (Kurozawa and Pavan, 2005; Marcuzzo et al., 2009).

Traditionally, chemical control of the bacterial spot has been performed with antibiotics and copper based products. However, the indiscriminate use of these products by farmers can induce the emergence of resistant populations of bacteria, contributing to these agrochemicals inefficiency (Lopes and Quezado, 2000).

The use of resistant cultivars as the most effective process to control plant diseases is cited by several authors (Quezado-Duval and Camargo, 2004; Silva-Lobo et al., 2005), who attribute to genetic resistance a strategy that reduces contamination of the environment and food by the use of agrochemicals (Lopes and Ávila, 2002).

Resistance genes *Bs1*, *Bs2* and *Bs3* have been used effectively in studies with different commercial cultivars of sweet pepper for resistance to bacterial blight for a short time. These three genes were identified respectively in the following accessions: PI 163192 (*C*. *annuum*), PI 260435 (*C. chacoense*) and PI 271322 (*C*. *annuum*) (Vallejos et al., 2010).

From the Early California Wonder variety, the isogenic lines ECW10R, ECW20R and ECW30R were created, which have the genes *Bs1*, *Bs2* and *Bs3*, respectively. These almost isogenic lines are resistant to different races of the pathogen. The ECW10R confers resistance to races 0, 2 and 5; ECW20R is resistant to races 0, 1, 2, 3, 7 and 8, while ECW30R has resistance to races 0, 1, 4, 7 and 9. PI 235047 (*Bs4*) has resistance to races 0, 1, 3, 4 and 6 (Stall et al., 2009). None of these has resistance to race 10.

In Brazil, Riva et al. (2004) identified three recessive genes that control resistance to *X. euvesicatoria* in *Capsicum* and Silva et al. (2017) indicated that a minimum of five recessive genes are responsible for resistance to bacterial spot in pepper.

3.4. Analysis of Generations with Likelihood

In genetic studies of generations it is possible to quantify the magnitude and nature of the genetic variability available in the segregating population, and to evaluate the relative importance of the gene effects that constitute the means of the populations studied. Simultaneous study of variances and population averages makes it possible to carry out generation tests involving parents (P_1 and P_2) and F_1, F_2 and backcross generations BC_1 (F_1 X P_1) and BC_2 (F_1 X P_2) (Cruz et al., 2014).

The study of variances is based on the estimation of genetic parameters such as heritability, phenotypic, genotypic and environmental variances, dominance, overdominance, additivity, among others. One of the major goals when using this approach is to quantify the magnitude and nature of genetic variability available in the segregating population (Moreira, 2006; Cruz et al., 2014).

There are different methods of analysis for the study of the genetic inheritance of the quantitative characteristics, in which it is possible to estimate the components of the mean, to calculate the means and genotypic, phenotypic and environmental variances of each generation, heritabilities in the broad and restricted sense, of dominance of the trait, to estimate the number of genes (Castle, 1921; Mather and Jinks, 1971). However, this method shows only the number of genes that express dominance (Cruz et al., 2012), does not identify the existence of genes of greater effect and/or polygenes interfering in the control of the characteristic.

With the aid of the maximum likelihood analysis, it is possible to identify this gene effect, considering hierarchical models, with the most general model: a gene of greater effect and polygenes with additive and dominance effects, including equal environmental variances in all generations. Independent genes of greater effect are also admitted as well as polygenes. The maximum likelihood allows for each model tested to estimate the genetic parameters and compose tests of interest considering the various hypotheses (Silva, 2003; Rezende, 2004).

3.5. Selection Index Mulamba and Mock

The genetic breeding programs of *C. annuum* L. aim to obtain cultivars with resistance to the main diseases, without neglecting the characteristics of commercial interest, considering a set of characteristics simultaneously. However, it is known that each characteristic to be improved has a certain complexity, since it is influenced by several factors such as genetic control and the physiological and environmental aspects (Tavares et al., 1999).

The practice of simultaneously evaluating a series of attributes in a single genotype, in order to select genotypes that exceed the commercial cultivar in the production and fruit quality aspects, is performed by the Selection Index.

The selection indices are a combination of several characteristics that aim to obtain answers to the selection, thus enabling the improvement of these characteristics together, even if there is no correlation between them, in order to obtain a linear function of the phenotypic values for different characters (Smith, 1936; Hazel, 1943).

There are indices that usedas economic weights genetic, phenotypic and economic covariance of these traits (Smith, 1936; Hazel, 1943; Williams, 1962); others that consider the minimum acceptable value for each character (Subandi et al., 1973), which involve the sum of the ranks of the genotypes for each of the characters (Mulamba e Mock, 1978); those obtained as a function of the heritability of the considered character (Smith et al., 1936), or even that use the differential of selection, to ponder the characters (Pesek and Baker, 1969).

The basis of the Mulamba and Mock index (1978) is a ranking and consists of ordering the genotypes in relation to each of the characteristics, according to the interest of the breeder. Subsequently, the notes are summed based on the multiple characters (Teixeira et al., 2012). The choice of this index is due to the ease of interpretation because it is a non-parametric index, thus, it does not require economic weights, estimation of parameters and averages.

Among the available indices, Mulamba and Mock are widely used in genotype selection in several crops such as papaya (Vivas et al. 2013), popcorn (Freitas et al., 2013), *Capsicum* (Oliveira et al., 2015), (Carias et al., 2016), soybean (Leite et al., 2016).

4. MATERIAL AND METHODS

4.1. Inheritance of bacterial spot resistance in *Capsicum annuum* var. *annuum*

4.1.1. Genotypes and generations

Generations F_1, F_2, BC_1, and BC_2 originated from crossing between two genotypes of *C*. *annuum* var. *annuum*, identified as UENF 2285 (female parent) and UENF 1381 (male parent) (Figure 1), both from the UENF germplasm bank. UENF 2285 is a variety of sweet pepper with squared fruit, which is susceptible to bacterial spot. UENF 1381 is a chili pepper (pungent) that has been used as a source of resistance to bacterial spot in the *Capsicum* breeding program developed by UENF.

Seedlings of all generations were produced at the UENF, in Campos dos Goytacazes, RJ, Brazil (21° 19' 23" S latitude and 41° 19' 40" W longitude). This stage was carried out from December 2014 to April 2016. Seedlings were sown in 128-cell polystyrene trays, remaining in a growth chamber at 28 °C. After the seedlings reached four to five leaves, they were transplanted to 500-mL pots containing a mixture of soil, sand, and manure (1:1:1 volume ratio). Hereafter, plants were left in greenhouse, and crop handling followed recommendations by Filgueira (2012), with some adaptations for this environment.

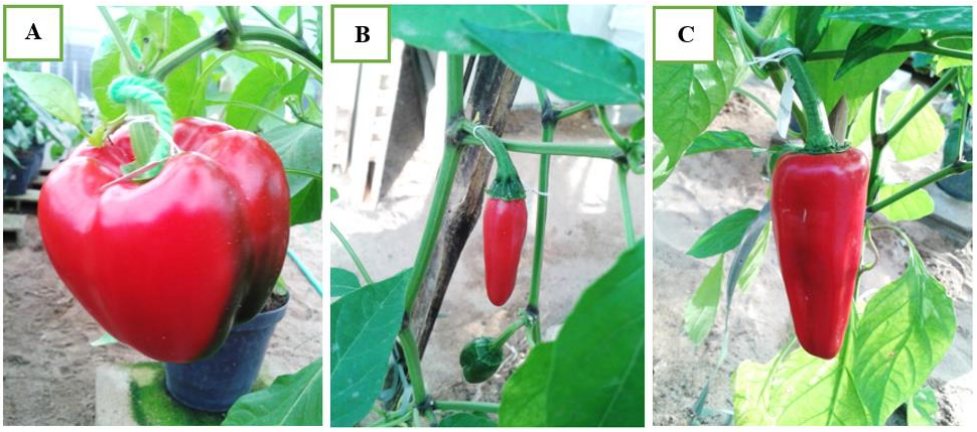

Figure 1. Fruit phenotype of *C. annuum* var. *annuum* parents and hybrid: A) UENF 2285, female parent, bacterial spot susceptible; B) UENF 1381, male parent, bacterial spot resistant; and C) F_1 hybrid from UENF 2285 x UENF 1381. Campos dos Goytacazes-RJ, UENF, Brazil (2016).

The crossings were performed early in the morning or late afternoon when buds were at the pre-anthesis. Female parent buds were emasculated and identified with a wool cloth. For pollen extraction from male parents, flowers were collected in the morning and dried under fluorescent bulbs. Afterward, pollen was removed and transferred into a gelatin capsule, being stored inside amber bottles with silica gel, in a refrigerator at ± 5 ºC, for later manual pollination. Emasculated flowers were pollinated and covered with paper bags to avoid further contamination.

Eighty crossings between UENF 2285 x UENF 1381 were carried out resulting in 24 hybrid fruit. Backcrosses were performed using 117 artificial crosses between UENF 2285 x F_1 (BC_1) and 66 between UENF 1381 x F_1 (BC_2). For that, ten plants of each parent and from hybrids were used, resulting in 101 fruit from BC_1 and 37 from BC_2. For F_2 generation, 253 self-fertilizations were made in F_1 generation, providing 11 fruit (Figure 2).

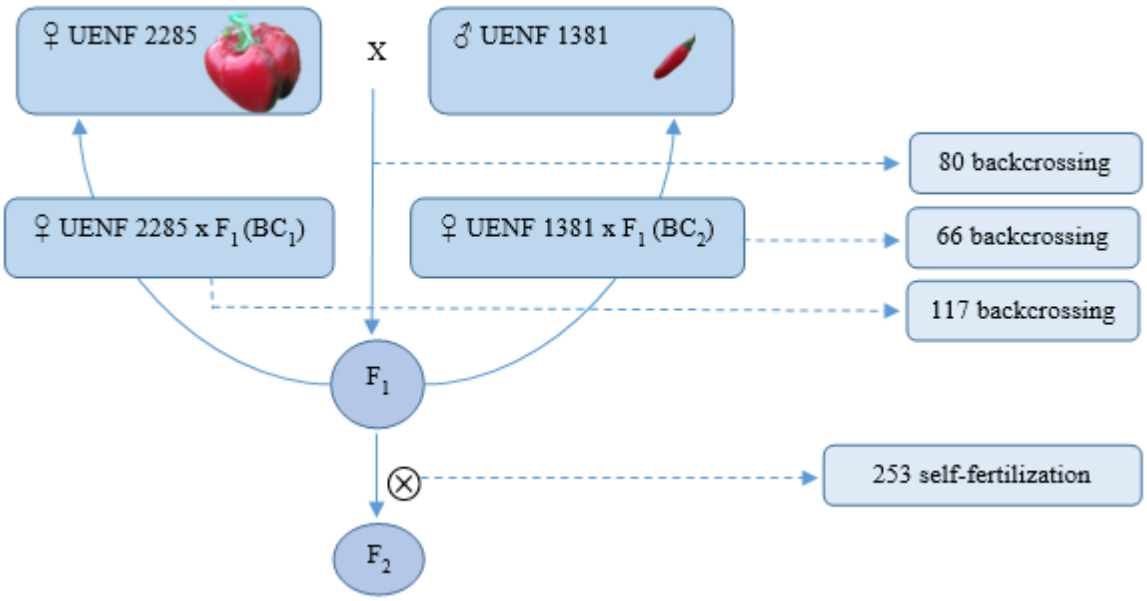

Figure 2. Flowchart depicting the *C. annuum* population development from crosses between UENF 2285 (bacterial spot susceptible) and UENF 1381 (bacterial spot resistant). Campos dos Goytacazes-RJ, UENF, Brazil (2016).

4.1.2. Bacterial spot inoculation and resistance assessment

Twenty plants of P_1, P_2, and F_1, plus 200 plants of F_2 and 40 plants of each backcross were used to evaluate resistance to bacterial spot. The bacterial strain ENA 4135, which was characterized by Riva et al. (2004) based on the differentiating genotypes proposed by Jones et al. (1998), was inoculated. The water-preserved strain (Castellani, 1939) was recovered in DYGS (Rodrigues Neto et al., 1986) liquid medium under agitation, for 36 hours at 28 °C. Thereafter, bacterial suspensions were transferred with Drigalsky's loop to Petri dishes containing solid DYGS medium. After 36 hours in a bacteriological incubator (28 °C), bacterial colonies were suspended in sterile water and cell concentrations adjusted to 10^8 cfu mL^{-1}, at 600 nm and 0.300 absorbance (Aguiar et al., 2000). Such concentration was applied for qualitative analysis to evaluating the hypersensitivity reaction (HR). Then, suspension (10^8 cfu mL^{-1}) was subjected to serial dilution in distilled water to reach a concentration of 10^5 cfu mL^{-1}, for quantitative resistance evaluation.

Inoculation was performed 38 days after transplanting, in two leaves of the plant upper middle third, by infiltration of a bacterial suspension with the above-

mentioned concentrations, in 1.0 cm^2 of the mesophyll (Riva et al., 2004). HR was assessed 24 and 48 hours after inoculation; it was solely considered the presence or the absence of an immediate and drastic cellular response of plants in contact with the pathogen.

Reaction to bacterial spot was quantitatively ascertained by means of a score scale. The scores consisted of: 1 - no visible symptoms, 2 - spotted chlorosis, 3 - yellowish leaves with some necrotic spots, 4 - necrotic spots, and 5 - total necrosis (Figure 3). This evaluation started five days after inoculation and lasted for seven days. In the end, scores below 2 were classified as resistant plants, and those above 2 were as susceptible (Riva-Souza et al., 2009).

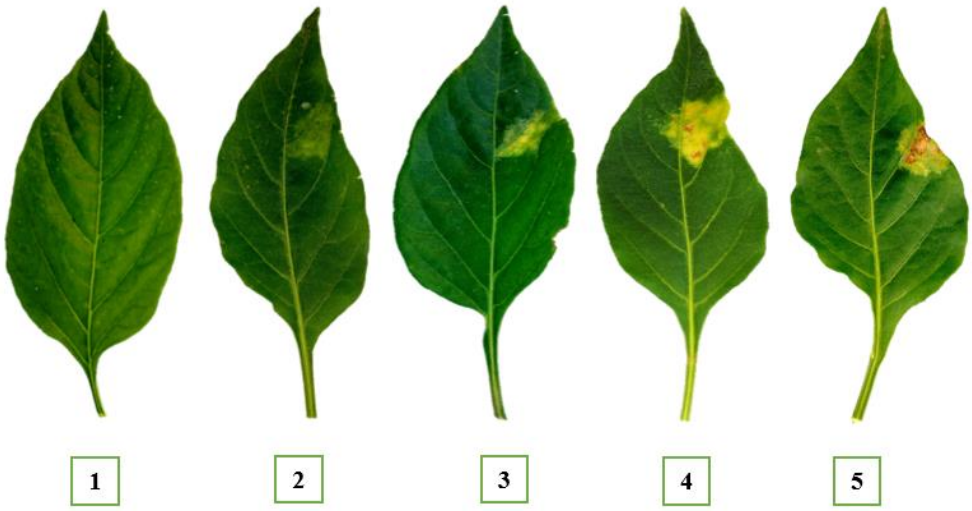

Figure 3. Rating scale for the assessment of bacterial spot severity (*X. euvesicatoria)* in *C. annuum* var. *annuum* leaves. Campos dos Goytacazes-RJ, UENF (Brazil), 2016.

Original rating was used to calculate the area under disease progress curve (AUDPC) as proposed by Shaner and Finney (1977):

$$AUDPC_i^{n=1} = \Sigma\,[x_i + (x_i + 1)\ x\ 0{,}5]\ x\ [(t_i + 1) - t_i],$$

In which:

n is the number of assessments; x_i is the disease incidence or severity; $[(t_i + 1) - t_i]$ is the interval between consecutive evaluations.

4.1.3. Analysis of variables

A quantitative approach was used to assess the results. This analysis was based on AUDPC analysis means and variances of the parental generations (F_1, F_2, BC_1, and BC_2). Each generation means were analyzed according to additive-dominant model, wherein averages varied only due to homozygosis (m), additive effect (a), and dominance deviation (d). Genetic parameters were estimated by weighted least squares.

From each generation variance analysis, the following estimates were obtained for the AUDPC results (Cruz, 2013):

- Environmental variance $(\sigma^2_{we}) = \sigma^2_{P_2}$;
- Phenotypic variance $(\sigma^2_f) = \sigma^2_{F_2}$;
- Genotypic variance $(\sigma^2_g) = \sigma^2_{F_2} - \sigma^2_{we}$;
- Additive variance $(\sigma^2_a) = 2\sigma^2_{F_2} - (\sigma^2_{BC_1} + \sigma^2_{BC_2})$;
- Broad-sense heritability $(h^2_b) = \frac{\sigma^2_g}{\sigma^2_{F_2}}$;
- Narrow-sense heritability $(h^2_n) = \frac{2\,\sigma^2_{F_2} - (\sigma^2_{BC_1} + \sigma^2_{BC_2})}{\sigma^2_{F_2}}$;
- Minimum number of genes involved in character determination $(\eta) = \frac{R^2}{8\sigma^2_g}$ (F_2); being R^2 is the total amplitude in F_2;
- k = Average Degree of Dominance (ADD) $= \frac{d}{a}$, in which: $\sigma^2_{P_2}$ is the P_2 variance; $\sigma^2_{F_1}$ is the F_1 variance; $\sigma^2_{F_2}$ is the F_2 variance; $\sigma^2_{BC_1}$ is the BC_1 variance; $\sigma^2_{BC_2}$ is the BC_2 variance.

  Statistical analyses were performed using the Genes program (Cruz, 2013).

4.2. Characterization of the resistance to bacterial spot in *Capsicum annuum* var. *annuum* by the method of analysis of generation with likelihood

4.2.1. Genotype, generations, experimental conditions and resistance evaluation

The genotypes and the generation of the genotypes used for this analysis as well as the conditions of plant cultivation, inoculation and evaluation of the

reaction to the bacterial spot are described in topic 4.1.1. The selection was made based on the F_2 generation.

4.2.2. Analysis of genetic inheritance by the maximum likelihood method

The methodology proposed by Silva (2003) was used to model and estimate parameters related to the effect of major gene and polygenes considering the maximum likelihood method, according to Silvera et al. (2015), Menezes (2015) and Batista et al. (2017). Based on the mean and variance components (Mather and Jinks 1982), as follows:

$P_1 = N(\mu-[a]-A, V_E)$

$P_2 = N(\mu-[a]+A, V_E)$

$F_1 = N(\mu-[d]-D, V_E)$

$$F_2=\frac{1}{4}N\left(\mu+\frac{[d]}{2}-A, V_E+V_A+V_D\right)+\frac{1}{2}N\left(\mu+\frac{[d]}{2}+D, V_E+V_A+V_D\right)+\frac{1}{4}N\left(\mu+\frac{[d]}{2}+A, V_E+V_A+V_D\right)$$

$$BC_1=\frac{1}{2}N\left(\mu+\frac{[a]}{2}+\frac{[d]}{2}-A, V_E+\frac{[VA]}{2}+V_D-S_{AD}\right)+\frac{1}{2}N\left(\mu-\frac{[a]}{2}+\frac{[d]}{2}+D, V_E+\frac{[VA]}{2}+V_D-S_{AD}\right)$$

$$BC_2=\frac{1}{2}N\left(\mu+\frac{[a]}{2}+\frac{[d]}{2}+A, V_E+\frac{[VA]}{2}+V_D+S_{AD}\right)+\frac{1}{2}N\left(\mu+\frac{[a]}{2}+\frac{[d]}{2}+D, V_E+\frac{[VA]}{2}+V_D+S_{AD}\right)$$

Where: μ = reference constant; A = additive effect of the major effect gene; D = effect of dominance of the major effect gene; [a] = additive polygenic component; [d] = polygenic component of dominance; V_A = additive variance; V_D = variance attributed to the dominance deviations of the polygenic effects; V_E = environmental variance; S_{AD} = component of the variation related to the products of the additive polygenic effects by the polygenic effects of dominance.

The density function for F_2 consisted of a mixture of three normal distributions, and the density function BC_1 and BC_2 consisted of a mixture of two normal distributions, and in each component of the mixture the mean variance components of polygenes do not change, changing only the effects of the major effect gene. All parameters were estimated using the maximum likelihood method and several genetic models were constructed (Table 1).

Table 1. Inherited models tested for resistance to bacterial spotting in generations of *C. annuum* var*. annuum*. UENF, Campos de Goytacazes-RJ, 2018.

Modelos	**Parâmetros**
1. Larger gene with additive and dominance effect + polygenes with additive and dominance effect	μ, A, D, [a], [d], V_A, V_D, S_{AD},V_E
2. Larger gene with additive and dominance effect + polygenes with additive effect only	μ, A, D, [a], V_A,V_E
3. Larger gene with additive effect only + polygenes with additive and dominance effect	μ, A, [a], [d], V_A, V_D, S_{AD},V_E
4. Larger gene with additive effect only + polygenes with additive effect only	μ, A, [a], V_A,V_E
5. Polygenes with additive and dominance effect	μ, [a], [d], V_A, V_D, S_{AD},V_E
6. Polygenes with additive effect only	μ, [a], V_A,V_E
7. Larger gene with additive and dominance effects	μ, A, D,V_E
8. Larger gene with additive effect only	μ, A,V_E
9. Environment effect only	μ,V_E

The likelihood tests were performed using the LR statistic (Modd et al., 1974) given by: $LR= 2ln \frac{L(Mi)}{L(Mj)}$, where the = *L(Mi) e L(Mj)* represent the likelihood functions of models i and j, where model i must be hierarchical to model j. The tests were performed using the Monogen program v.0.1 (Silva, 2003).

4.3. Selection of genotypes resistant to bacterial spot in a segregating population of *Capsicum annuum* var. *annuum*

4.3.1. Genotype, generations, experimental conditions and resistance evaluation

The genotypes and the generation of the genotypes used for this analysis as well as the conditions of plant cultivation, inoculation and evaluation of the reaction to the bacterial spot are described in topic 4.1.1. The selection was made based on the F_2 generation.

4.3.2. Agronomic attributes phenotyping

Nine agronomic attributes were phenotyped according to the following descriptors:

1. Fruit length (FL) - Determined in the longitudinal region of the fruits, with the aid of a digital caliper, in an average of five mature fruits, per plant, in millimeters (mm);

2. Fruit diameter (FD) - Determined in the equatorial region of the fruits, using a digital caliper, in an average of five ripe fruits, per plant, in millimeters (mm);

3. Pericarp thickness (PT) - Determined by the thickness of the pericarp of the fruits, using a digital caliper, in an average of five ripe fruits, per plant, in millimeters (mm);

4. Fruit format (FF) - Determined according to the specific descriptors for *Capsicum* spp. of Bioversity International (IPGRI, 1995);

5. Presence or absence of capsaicin (CAPS) - determined by the presence or absence of pungency through the reaction of substances present in the placenta with ammonium vanadate, as described by Derera (2000), modified by Riva (2004).

6. Soluble solids content (SSC) - Quantified using digital refractometer, five mature fruits per plant, in ° Brix;

7. Vitamin C (VITc) - Quantified by means of titration with 2.6-dichlorophenol indophenol, five mature fruits per plant in milligrams;

8. Titratable acidity (TA) - Quantified by titration with 0.1 M sodium hydroxide solution, five mature fruits per plant in milligrams.

9. Area Under the Disease Progression Curve (AUDPC) - Obtained by transforming the scale values to obtain the AUDPC average.

4.3.3. Statistical analysis

The analysis of variance and estimation of the genetic parameters of the agronomic and resistance characteristics were evaluated separately, using the Genes program (Cruz, 2013).

To select the best individuals for the continuation of the plant breeding program of *C. annuum* var. *annuum*, the Mulamba and Mock index (1978) were used, also using the Genes program (Cruz, 2013). In order to obtain chili with resistance to bacterial blight, 30% of 188 plants of the F_2 generation were

selected, assigning higher weights, weight 50 for AUDPC, length and diameter of the fruit and for the other characteristics, weight 20.

4.4. New genes responsible for hypersensitive response in *Capsicum annuum* when inoculated with *Xanthomonas gardneri*

To verify the presence of the *bs5* and *bs6* genes in UENF 1381 plants, two trials were conducted under greenhouse conditions from May to September 2017 at the Plant Pathology Department of the University of Florida in the city of Gainesville, Florida, USA.

4.4.1. Plant Material

The parents UENF 2285 (P_1) x UENF 1381 (P_2), both belong to the species *C. annuum* var. *annuum* and the F_1 and F_2 generations from this crossing were used in that assay. UENF 2285, a sweet pepper variety, is considered a susceptibility standard in studies for several diseases (Wai et al., 2015; Silva et al., 2017). In addition, the male parent is a pungent pepper, which is resistant to bacterial blight (Costa et al., 2002; Bento et al., 2017) (Figure 4).

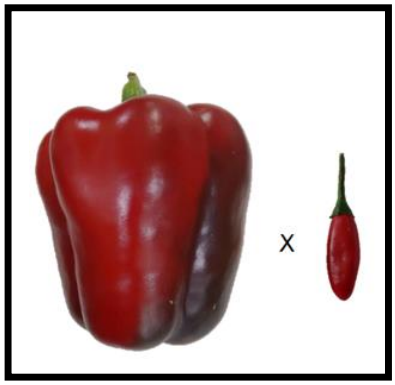

Figure 4. Parental UENF 2285 (susceptible to bacterial stain) and UENF 1381 (resistant), used as parents, female and male, to obtain the F_1, F_2 generations.

4.4.2. Experimental Conditions

In the first trial, the experimental design was completely randomized to nine parent plants (three P_1 and six P_2), six F_1 plants and 60 F_2 plants, three ECW 50R

plants, three ECW 60R plants, as control plants with the genes *bs5* and *bs6*, totalizing 81 plants (Figure 5). The aim of this experiment was to verify the presence of genes *bs5* and *bs6*, in resistant parental and in F_2 genotypes.

In the second trial, only the parents and the F_2 generation were sown, and the experimental design was completely randomized to 12 plants of the parents (six of each parent) and 96 F_2 plants, totalizing 108 plants. In this essay #2, it was intended to confirm the results of the first experiment.

For sowing, aluminum trays containing commercial substrate were used. When the seedlings were with four leaves, the transplant was performed for plastic containers with a capacity of two liters. Irrigation was performed once a day, with water replenishment according to water demand.

Figure 5. A) Transplanting of the seedlings to pots in a greenhouse. B) Method of inoculation of the bacterial suspension by infiltration and C) Evaluation of the experiment, in which white label corresponds to *X. euvesicatoria* in the concentration of $2x10^3$ and blue labels to *X. gardneri* in the concentration of $2x10^8$.

4.4.3. Molecular analysis to identify recessive resistance genes *bs5* and *bs6*

The plant material for the molecular analysis was obtained from seedlings of the parents *C. annuum* (UENF 1381 and UENF 2285). PI 235047 (*C. pubescens*) and tomatoes of the variety Bonny Best were used as controls, since both have the *Bs4* resistance gene.

To extract DNA from the C. *annuum* genotypes, the extraction buffer (0.35 M sorbitol, 0.1 M tris base, 5 mM EDTA, pH 7.5) of nucleotides (Tris 0.2 M, 0.05 M EDTA, 2 M NaCl, 2% CTAB) and microprep (2.5 ml of the extraction buffer, 2.5 ml of the nucleotide buffer and 1.0 ml of Sarkosyl 5%) (Fulton et al., 1995) were provided.

Genomic DNA was extracted from leaves using a protocol proposed by Fulton et al. (1995). Fifty to 100 mg of leaf tissue (approximately 4-8 young leaves, up to 1.5 cm in length) was collected and placed in the bottom of a 1.5 ml Eppendorf tube. The microprep buffer was prepared and kept in room temperature. The leaflets were grounded in 700 µL of the microprep buffer, followed by the addition of 550 µL of buffer. The suspenssion was then mixed by *Vortex*. The samples were incubated in water bath at 65 ºC for 30-120 minutes. After the incubation, 700µL of chloroform:isoamyl (24:1) was added to the tube. The tubes were mixed one by one, followed by centrifugation at 15,000 rpm for 5 minutes. At this step, a top layer was formed in the top of the tubes, which was pipetted to a new tube (approximately 400 µL). Isopropanol was kept in ice and it was added (240µL) to the tubes. The tubes were then carefully inverted for DNA precipitation, followed by immediate centrifugation at 15,000 rpm for 5 minutes. Isopropanol was added to the tubes and the DNA wash was performed with 70% ethanol. The tubes were placed upside down on a paper towel for 10 minutes to dry the *pellet*. The DNA was ressuspended in 100 µL of TE + RNAse and incubated at 65 ºC, for 15 minutes, followed by centrifugation at 10,000 rpm for 10 minutes. The DNA was stored at 4 ºC up to a week.

In the PCR reaction 1 µL of DNA was used. Specific primers were tested for the *bs5* and *bs6* genes (unpublished data and under patent secrecy) and also for the *Bs4* and *Bs4C* genes for identification of these genes in the working population. The following primers were used to identify *Bs4* and *Bs4C*:

Bs4: A03F (GGGTTGGAGTCCGAAGAGCAGG) and B03R (GACTAACCAACGCAAGTTATTGGACAGG);

Bs4C: 12600F2 (CTCTACAATATTTCCAGCAGTTAGC) and 12600R1 (GCTTTACTCAAACATACAAGTGAC).

The size of the PCR products for the *Bs4* and *Bs4C* genes is 535bp and 450bp, respectively. Identification of the presence or absence of the *Bs4*, *Bs4C*,

bs5 and *bs6* genes was done by checking the band pattern of the primers specific to each gene. PCR products were analyzed on 3% agarose gel.

4.4.4. Phenotyping of hypersensitivity reaction (HR) in plants of UENF 1381

The UENF1381 accession plants were phenotyped for the HR, using different races *X. euvesicatoria* and *X. gardneri* to identify whether or not the bacterial isolates has the genes *avrBs1*, *avrBs2* and *avrBs3*.

Two isolates of different species of *Xanthomonas* were tested. The ENA 4135 isolate, characterized in previous tests as race T1P3 of *X. axonopodis* pv. *vesicatoria* (Riva et al., 2004), later renamed to *X. euvesicatoria* and an isolate of *X. gardneri* (race 444). The reaction to *X. cynarae* in the plant population was also tested. Of the three species cited, *X. euvesicatoria* is the one that occurs in Brazil.

Bacteria were grown in liquid agar and 50% glycerol medium without antibiotic. After a period of 36 hours of growth in a bacteriological oven at 28 ° C, the bacterial colonies were suspended in sterile water and their cell concentration adjusted to 10^3 cfu mL^{-1}, using a spectrophotometer using the wavelength of 600 nm and optical density of 0.300 to obtain the concentration of 2.0 x 10^8 cfu mL^{-1}.

The inoculation was carried out at 21 days after transplantation, in one of the leaves of the upper middle third of the plant, by infiltration of bacterial suspension (Figure 2B), in the concentration of 10^3 cfu mL^{-1}, in 1.0 cm^2 of the mesophyll (Bongiolo Neto et al., 1986; Juhász, 2002; Costa et al., 2002; Sudré, 2003; Riva et al., 2004). They were also inoculated with the concentration of 10^8 cfu mL^{-1} to identify HR.

4.4.5. Resistance of UENF 1381 to different isolates of *Xanthomonas* spp.

Five plants of accessions UENF 1381 and UENF 2285 of *C*. *annuum* were used to test the virulence of two isolates of *X. euvesicatoria* (ENA 4135 and 18b), one isolate of *X. gardneri* (444), one of *X. perforans* (2010) and one of *X. vesicatoria* (143). In addition, reaction to these races was observed in UENF 1381, since the resistance described in this accession until now is only for *X. euvesicatoria*.

For the analysis of the variables, the null hypothesis (H_0) can be accepted or not by comparing the values of χ^2 calculated with the values of χ^2, with 5% of

significance and the degree of freedom equal to K-1. The analyzes were performed in the GENES software (Cruz, 2013).

To determine the growth of bacteria in the genotypes, suspensions were used in sterilized water containing 3×10^5 cfu mL^{-1} of the strains, which were infiltrated in *Capsicum* leaves. The inoculated plants were incubated in a growth room at 25-28 °C for 10 days (Schornack et al., 2008; Hu et al., 2013). In each plant three leaves were inoculated with five inoculation areas, corresponding to the five bacterial isolates used (Figure 6A and 6B).

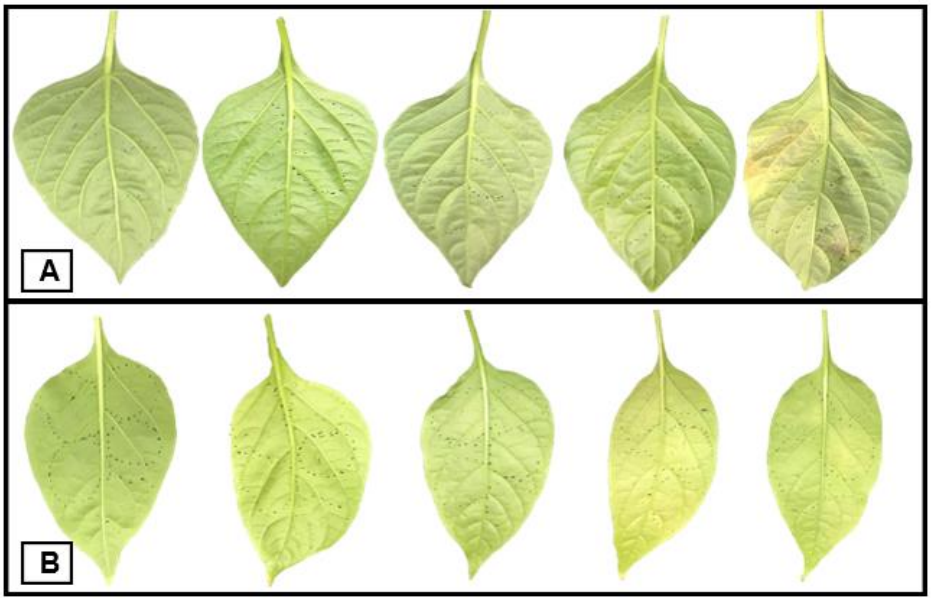

Figure 6. A) Sample of leaves of accesses UENF 2285, B) UENF 1381, on days 0, 2, 4, 6 and 8, for bacterial population formation of *X. euvesicatoria* ENA 4135, *X. euvesicatoria* 18b, *X. gardneri*, *X. perforans* and *X. vesicatoria*, in Gainesville, 2017.

The bacterial populations were counted from a tissue sample of 1 cm^2 of inoculated leaf area, macerated in 1 mL of sterilized water, followed by serial dilution, which were scored on agar-nutrient plates. For each genotype, three leaves were inoculated, obtaining a triplicate of each isolate. Plates were incubated at 28 °C and colonies were counted to calculate leaf bacterial concentration for each sample (Figure 7).

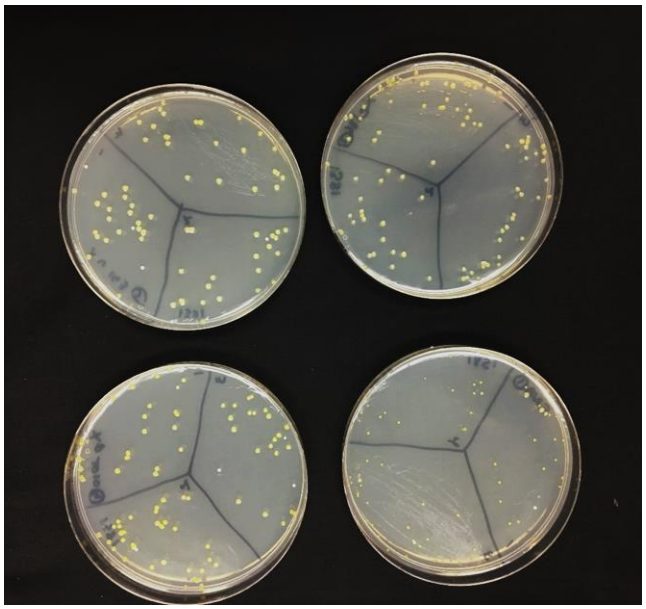

Figure 7. Plates with agar medium, on the 4th day of evaluation of the bacterial population of *X. euvesicatoria* 18b, *X. gardneri*, *X. perforans* and *X. vesicatoria*, nonparental UENF1381, of *C*. *annuum* var. *annuum* in Gainesville, 2017.

After inoculation of the bacteria in the five plants of each parent, a plant of each parent was selected, in which a disc of each inoculated leaf, of 1.3 cm^2 (two discs / leaf) was extracted. Immediately after, the disks were placed in test tubes. The bacterial suspension of each species was scored on agar plate and counting was performed every two days. For each species of *Xanthomonas*, the triplicate was made to obtain an average. The results obtained in the counts represented the number of colony forming units per cm^2 (cfu cm^2) of leaf limbus, whose values were transformed into log cfu cm^2. With these data, curves were constructed representing the evolution of the resident population of the bacterium over the evaluation period.

Inoculations were also carried out with five *X. vesicatoria* races: BA29-1-143, BA26-1-611, BA26-4-620, BA21-1-606 and BA21-4-607, and one strain of *X. perforans*: RR110-AUS14, in the parental UENF1381 and in an F_2 plant (#6) because they are promising in future research with *Xanthomonas*.

5. RESULTS AND DISCUSSION

5.1. Inheritance of bacterial spot resistance in *Capsicum annuum* var. *annuum*

After assessing inoculations with *X. euvesicatoria*, no hypersensitivity reaction (HR) was evident for all evaluated generations. Therefore, the interaction between plant and pathogen was compatible (Bergamin Filho and Amorim, 2002). From the scoring scale, the results showed that all P_1 parent plants reached an incidence of 100%, i.e., all plants were susceptible (Figure 8A). These findings corroborate those reported in preceding studies such as Moreira et al. (2013a) and Moreira et al. (2015). Moreover, P_2 had its resistance confirmed (Figure 8B), as already observed by Moreira et al. (2010), Moreira et al. (2013b) and Pimenta et al. (2016). It is noteworthy emphasizing that highly contrasting parents are essential for the evaluated characteristic. In F_1 generation, 100% of the plants developed susceptibility symptoms (Figure 8C), indicating a recessive genetic control for resistance.

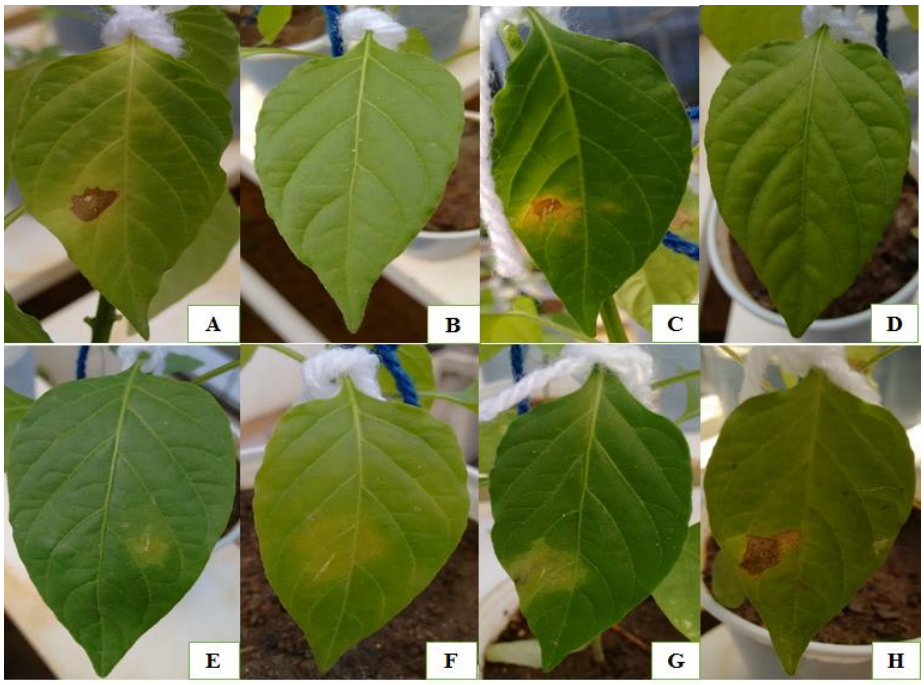

Figure 8. Reactions to bacterial spot infection in different *C. annuum* var. *annuum* plant generations, when inoculated with *Xanthomonas euvesicatoria* at 10^5 cfu mL^{-1}. A) P_1 (UENF 2285 - susceptible), B) P_2 (UENF 1381 - resistant), C) F_1 generation - susceptible (D, E, F, G, H), plants representative of F_2 generation. Campos dos Goytacazes-RJ, UENF (Brazil), 2016.

F_2 plants showed various symptoms (Figure 8 D, E, F, G, and H), characterizing a large genetic variability of this generation. Aggressiveness was noticed by a fast appearance of symptoms in susceptible plants. In BC_1 and BC_2, susceptibility rates were between 92.5% and 82.5%, respectively. These values indicate that a single gene might possibly control such a characteristic.

Resistance quantitative analysis was made considering the AUDPC values. P_2 mean (13.80) was lower than that of P_1 (26.30). It confirms this genotype resistance since the smaller the affected area, the more resistant is a genotype (Table 1). Riva-Souza et al. (2009) and Moreira et al. (2015), evaluating plants for the same time, also confirmed resistance by lower means for the same resistant parent, which were 15.67 and 15.5, respectively. Likewise, Demosthenes and Bentes (2011), evaluating resistance to bacterial wilt in of *Capsicum* accessions, reported plants with lower AUDPC, being classified as resistant. F_1, F_2, BC_1, and BC_2 showed AUDPC averages close to their susceptible parent (Table 2).

Table 2. Number of evaluated plants, averages and variances for the area under disease progression curve (AUDPC) of different generations from the crossing of UENF 2285 x UENF 1381 accessions of *C. annuum* var. *annuum*.

Generation	Number of evaluated plants	**AUDPC**	
		Mean (m)	Variance (σ^2)
P_1	20	26.30	9.62
P_2	20	13.80	0.85
F_1	20	22.98	19.38
F_2	200	21.17	30.46
BC_1	40	24.53	21.82
BC_2	40	20.43	23.94

F_2 reached a higher phenotypic variance regarding AUDPC (30.46), as already expected (Table 2). This generation receives greater influence from both genetic (σ^2_g) and environmental (σ^2_e) factors because of a high allelic combination between individuals. Therefore, σ^2_g and σ^2_e were 25.22 and 5.23, respectively (Table 2). In this case, genetic variation (sum of additive + dominance), which is important to estimate inheritability, registered a higher value than the environmental one. Moreira et al. (2015), studying *C. annuum* recombinant inbred lines, also observed σ^2_g values higher than σ^2_e ones (172.4 and 17.1, respectively), showing that genotype has more influence than the environment on the expression of AUDPC.

There was transgressive segregation in F_2 generation, with a maximum value of 33.0 and a minimum of 9.5 (Table 3). These values are outside the upper and lower patterns of the parents, evidencing that more than one gene controls the resistance to *X. euvesicatoria*.

The variances of the assessed genetic parameters suggested that more than four genes control this bacterial resistance inheritance (Table 3). The greater the number of genes involved in controlling an specific character, the higher the number of genotypic combinations within a population and generations are required to achieve full homozygosity (Baldissera et al., 2014).

Jones et al. (2002) and Riva et al. (2004) assessed different *Capsicum* genotypes and identified two (*bs5* and *bs6*) and three recessive genes, respectively. Lobo et al. (2005), evaluating the same disease in tomato

accessions, noted that the number of genes ranged from four to eight, indicating a polygenic inheritance, based on the used genotypes and crossing combinations among them. As the number of genes increased, there is a rise in the number of phenotypes, reducing the differences among them. F_2 segregation tends to a continuous distribution. It also reduces each allele contribution to a given character.

Broad-sense heritability (h^2_b) was 82.81%, and the narrow-sense one (h^2_n) was 49.74% (Table 3). It means that nearly 83% of the total variance in F_2 in under genetic control and about 50% comes from additive genetic effects. Riva et al. (2004) evaluated inheritance of bacterial spot resistance in *Capsicum* from crossing between a susceptible parent and a resistant one (UENF 1381). These authors verified broad- and narrow-sense heritability with values close to those found here, being of 82, 54 and 50.17%, respectively.

Heritability is dependent on genetic and environmental variances. Several genes rule this characteristic and the environmental influence tends to be quite high. However, for both above-cited studies, with polygenic inheritance, both genetic and additive effects were majorly expressive. Concerning breeding purposes, this is relevant because the results indicate that variation has no influence from the environment. Thus, a character that has high heritability, facilitates and maximizes the achievement of selection gains in a breeding program (Gonçalves et al., 2003).

There is a trend of h^2_b being higher than h^2_n, since the first reflects both additive and non-additive variances, while the second considers only the additive component. Studying the inheritance of resistance to tomato blight, Abreu et al. (2008) observed values of 54.86% and 9.06% for h^2_b and h^2_n, respectively; hence, heritability values were lower. It highlights a most intense environmental influence on tomato blight than it was on the bacterial spot.

Genotypic variance has to be studied for breeding programs to be successful. Knowing the genotype variations allows us to understand the importance of certain genetic factors for a given population (Amaral et al., 1996). By means of this variable, three major components can be measured (Fisher, 1918): additive variance (mean effects of genes), dominance (interaction between alleles within the same locus), and epistatic (interaction between alleles at different loci).

Table 3. Estimates of genetic parameters based on AUDPC values to evaluate resistance to bacterial spot (*X. euvesicatoria*) in genotypes of different generations from crosses between *C. annuum* var. *annuum* accessions (UENF 2285 and UENF 1381).

Genetics Parameters	Estimates of bacterial spot resistance
Phenotypic variance (σ^2_f)	30.46
Environmental variance (σ^2_e)	5.23
Genotypic variance (σ^2_g)	25.22
Additive variance (σ^2_a)	15.15
Variance of dominance (σ^2_d)	10.07
Broad-sense heritability (h^2_b%)	82.81
Narrow-sense heritability (h^2_n%)	49.74
Average degree of dominance (ADD)	0.47
Maximum value in F_2	31.00
Minimum value in F_2	11.50
Minimum number of genes (η)	4.56
Genotypic determination (R^2)	86.36

Additive variance (15.15) was superior to dominance variance (10.07) (Table 3). These outcomes indicate a high covariance between progeny and its respective parents, what implies in possible selection gains. Additive variance is a key tool for breeders since it enables the selection of a most efficient breeding method for fixation of a characteristic of interest (Cruz, 2014). Riva-Souza et al. (2007), studying the same pathosystem, pinpointed dominance deviations (1.11) superior to additive effects (0.32), in this case, making the selection difficult. Although studying the same pathosystem, the estimates obtained for each genetic parameter for the same trait is unique because depends on the genetic of the parents.

Average Degree of Dominance (ADD) was 0.47, expressing that genotypic value of homozygous was lower than that of heterozygous, consisting of a partial dominance with prospective epistatic effect. Riva et al. (2004), studying the same pathosystem, identified an ADD of 1.13. Similarly, Bento et al. (2013), evaluating the resistance of *C. baccatum* to PepYMV, verified an ADD of 1.12, indicating an

overdominant action. Juhász et al. (2008) observed a value of 1.0 in a tomato-PepYMV pathosystem, indicating complete dominance. ADD is estimated by spotting a heterozygous position in relation to its contrasting homozygous parents; thus, ADD values may be different, even in a similar pathosystem.

By the coefficient of determination (R^2), gene effects on resistance to the bacterial spot were confirmed as additives. This effect is explained by 86.36% of the total variation (Table 3). Moreira et al. (2015), evaluating the same disease in *C. annuum* recombinant inbred lines, found R^2 higher than 90%, what was associated with the use of a late generation (F_7). With advanced generations, R^2 is associated to h^2_n because genotypes become pure lines; therefore, variability is attributed to an additive action, which undergoes duplication ($2\sigma^2_a$). High R^2 values result in higher accuracy in selection of superior lineages for resistance to bacterial spot, maximizing thus genetic gains (Ribeiro et al., 2009).

The mean generation analysis showed that mean (*m*), additivity (*a*) and dominance (*d*), were significant and that an additive-dominant model could explain genetic effects involved in the inheritance of resistance. Table 4 shows that the mean parameter was more estimative to explain the characteristic (19.90). Besides, the additive effect was higher (6.06) compared to the dominance effect (3.31) (Table 4), similar result was observed by Costa et al. (2002) in a similar pathosystem.

Table 4. Estimation of genetic effects for resistance to bacterial spot, in a partial model (*m, a, d*), for generations P_1, P_2, F_1, F_2, BC_1, and BC_2 from the crossing between UENF 2285 and UENF 1381 accessions of *C. annuum* var. *annuum*.

Parameter[1]	**AUDPC**		
	Estimative	Variance	T
M	19.90	0.11	59.66
A	6.06	0.11	18.22
D	3.31	0.61	4.24

[1]*m* – mean, *a* – additivity and *d* – dominance.

In Solanaceae have been observed the magnitude of the additive effect on the dominant resistance to bacterial spot, in different families of tomato (Lobo et

al., 2005) and *C. annuum* (Riva et al. 2004), similar to that found in this work. With this effect the fixation of the characteristic in future generations is possible because additivity is predominant in the genetic control of resistance to bacterial spot.

5.2. Characterization of the resistance to bacterial spot in *Capsicum annuum* var. *annuum* by the method of analysis of generation with likelihood

In terms of Area Under Disease Progression Curve (AUDPC) variation was observed in the different generations evaluated (Figure 9). The parents (P_1 and P_2) confirmed that they are contrasting for the bacterial stain resistance characteristic.

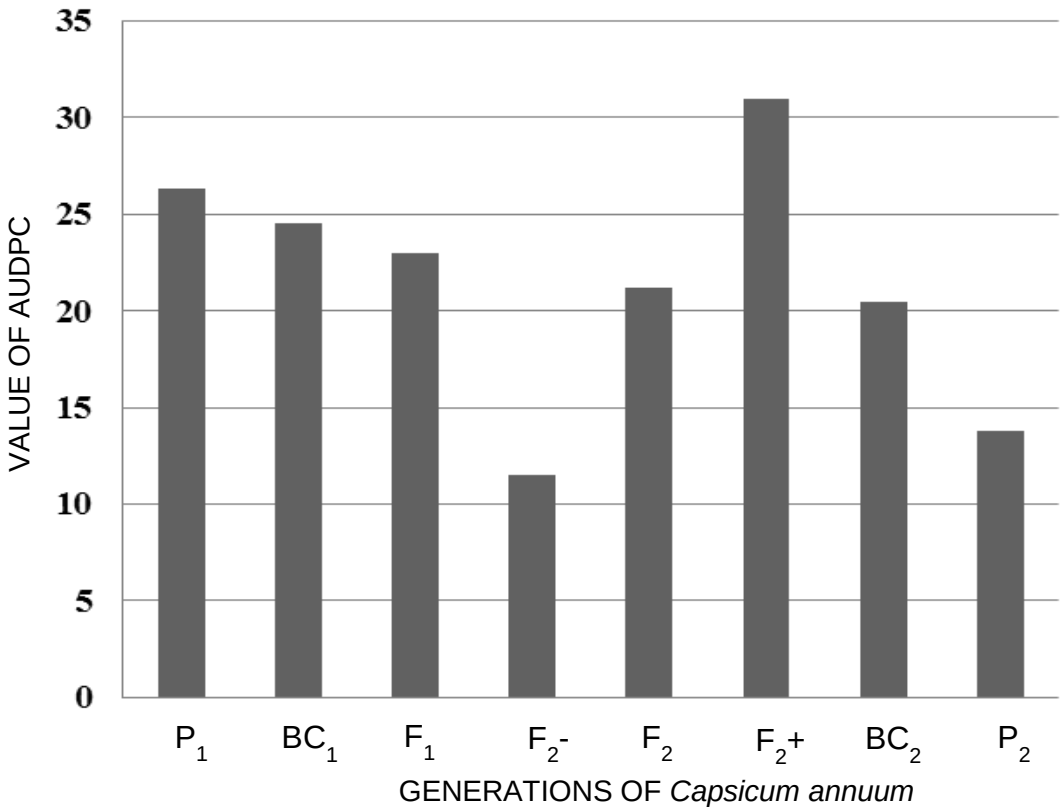

Figure 9. Averages for Area Under Disease Progression Curve (AUDPC) in relation to bacterial spot in different generations of the cross between UENF 2285 x UENF 1381 of *C. annuum*. UENF, Campos dos Goytacazes, RJ, 2018.

The AUDPC of the resistant father was less than 15, and the susceptible parent, greater than 25, thus, the higher the score, the higher the AUDPC. Moreira et al. (2015) working with the same pathological system, found values of 15.5 for UENF 1381 and of 54.3 for UENF 2285. Both backcrossing tended to the average of the observed scores for the respective parental, with BC_2 close to P_2 and BC_1 of P_1.

The presence of transgressive individuals was observed in F_2, where the average of some resistant and susceptible genotypes exceeded the average of the resistant and susceptible parent, respectively (Figure 9).

From the AUDPC, the genetic models were tested for the inheritance of resistance to bacterial spot, with the aid of the Likelihood Method, proposed by Silva (2003). As a population of polygenic inheritance, with the minimum number of five genes responsible for the control of resistance, with predominant additive effect (Silva et al., 2017), the comparison of the contrasts of the four models (Major gene with additive effect only + polygenes with additive effect only) with eight (Gene with additive effect only), that there are no significant differences, indicating the existence of a larger gene with additive effect only (Table 5).

Afterwards, models three (greater gene with additive effect only + polygenes with additive effect and dominance) and eight (major gene with additive effect only) were compared, this contrast being significant differences, indicating also the evidence of associated polygenes. Finally, the contrast of the three models (major gene with additive effect only + polygenes with additive and dominance effect) and the five (Polygenes with additive and dominance effect) presented significant differences, so the appropriate model is the three be the most complete (Table 5).

Silva et al. (2017), evaluating the inheritance of resistance to bacterial spot in generations of *C. annuum* var. *annuum*, the same used in this study, identified that the characteristic is quantitative, because the number of genes that govern the characteristic is of at least five genes. They also identified that the additive effect was more expressive than the dominant one. Thus, the model of this work complements these results, generating information that corroborates the published results, as it discriminates in a more detailed way the presence of additive genes and that there is influence of the dominance effect.

Similar results were found by Ferreira (2017) in the determination of genetic models by means of the likelihood function. This method showed that the inheritance of resistance to tomato blight is conferred by a larger gene, with additive and dominant effect plus polygenes, with additive effect plus environmental effects. Batista et al. (2017), to obtain common genotypes resistant to fusarium wilt, have identified that the resistance is governed by a dominant gene of greater effect and polygenes.

Table 5. Inheritance hypothesis tests by means of the maximum likelihood function for resistance to bacterial spot in generations of *C. annuum* var. *annuum* measured by the Area Under the Disease Progression Curve (AUDPC). UENF, Campos dos Goytacazes, RJ, 2018.

Models (1)	**DF**	**AUDPC to bacterial spot**	
		χ_c^2	**Probability**
1 vs. 2	3	(2)	(2)
1 vs. 3	1	97.46525	0.000000201
1 vs. 4	4	128.22049	0.000000618
1 vs. 5	2	98.06364	0.000000312
1 vs. 6	5	134.15992	0.000000444
1 vs. 7	5	(2)	(2)
1 vs. 8	6	128.68889	0.000000729
1 vs. 9	7	238.56991	0.000000875
2 vs. 4	1	138.55739	0.000000525
2 vs. 6	2	144.49683	0.000000457
2 vs. 7	2	(2)	(2)
2 vs. 8	3	139.02579	0.000000474
2 vs. 9	4	248.90682	0.000000803
3 vs. 5	1	0.59838	0.439196120**
3 vs. 6	4	36.69466	0.000000473
3 vs. 8	5	31.22363	0.000008749**
3 vs. 9	6	141.10466	0.000000743
4 vs. 6	1	5.93943	0.014805656
4 vs. 8	2	0.46839	0.791204053^{ns}
4 vs. 9	3	110.34942	0.000000529
5 vs. 6	3	36.09628	0.000000336
5 vs. 9	5	140.50627	0.000000472
6 vs. 9	2	104.40999	0.000000376
7 vs. 8	1	139.35740	0.000000374
7 vs. 9	2	249.23842	0.000000838
8 vs. 9	1	(2)	(2)

(1) Likelihood ratio tests, performed using the LR statistic, with the Monogen v 0.1 model genetic inheritance program (Silva, 2003). (2) Negative value, perhaps due to convergence problems.

Diniz (2016), evaluating the inheritance of resistance of *C. frutescens* to *M. enterolobii*, also using the tests of maximum likelihood, observed that in its population there was no evidence that there are polygenes with dominance effect. With the interpretation of the sets of test results it is possible to infer that the resistance is controlled by a recessive gene of greater effect, of additive effect only, different from the results of this work.

Naresh et al. (2016) studying the inheritance of Cucumber mosaic virus (CMV) resistance in *Capsicum* spp. Germplasm, verified that the resistance is of a recessive polygenic nature and that susceptibility in hybrids is associated with a non-allelic interaction in some crazy. As a function of the major effect gene, the identification of polygenes in general is possible with the maximum likelihood estimators (Silva, 2003).

Silva et al. (2009), in studies on the inheritance of parthenocarpy in zucchini, identified by the likelihood method that this character is of monogenic inheritance, being a main gene with partial dominance.

Breeding plants for the resistance of a particular disease controlled by many genes becomes more difficult. Quezado-Duval and Lopes (2010) reported that some tomato genotypes have shown resistance to different races of *Xanthomonas*, but this resistance is complex, because it has different genetic groupings with larger and smaller additive effects.

Pathogen variability is also a factor that brings additional challenge to breeders. The species *Xanthomonas* present different races, for example, in the case of *X. euvesicatoria*, for which 11 races have already been described (Minsavage et al., 1990; Ritchie et al., 1998; Sahin and Miller, 1998). Among these races, T1P3 was used to perform this work.

The research involving *Capsicum* and tomatoes has shown that resistance to bacterial blight is an inheritance complex trait (Riva et al., 2004; Silva et al., 2017) and because of its association with the variability of the pathogen, turns out to be a challenge for plant breeders in obtaining resistant cultivars to this disease.

5.3. Selection of individuals resistant to bacterial spot in a segregating population of *Capsicum annuum* var. *annuum*

The analysis of variance shows that the AUDPC, FL, PT, TA characteristics were significant at 5% probability (Table 6). The coefficient of variation (CVe) ranged from 10 to 35%.

The CVe is a measure of the variability of the experimental results, being relevant in determining the necessary repetitions in an essay, necessary to detect a difference between averages of treatments with a given probability. The CVs are associated with the residual error in the analysis of variance, thus making it possible to differentiate the means between the treatments and establish ranges of values that guide the researchers about the validity of their experiments (Pimentel-Gomes, 2009; Nesi et al., 2010).

Table 6. Phenotypic and phenotypic parameters of the area under the disease progression curve (AUDPC), fruit length (FL), fruit diameter (FD), pericarp thickness (PT), soluble solids content (SSC), titratable acidity (TA), vitamin C (VITc) in *C. annuum* var. *annuum*, evaluated in field experiment. Campos dos Goytacazes - RJ, Brazil.

FV	AUDPC	FL	FD	PT	SSC	TA	VITc
Medium Square	30.803	341.77	28.74	0.812	2.53	2.89	481.7
Genotype	5.68*	1.74*	0.5^{ns}	1.4*	0.33^{ns}	1.92*	0.96^{ns}
Phenotypic variance (σ^2_f)	30.80	341.76	26.27	0.74	4.25	2.89	429.84
Environmental variance (σ^2_e)	5.42	195.93	13.34	0.23	1.53	1.50	150.71
Genotypic variance (σ^2_g)	25.38	145.84	12.93	0.51	2.72	1.39	279.13
Heritability (H^2%)	82.41	42.67	49.20	69.31	63.65	47.95	64.94
Coefficient of variation (CV%)	11.15	22.83	25.75	21.95	10.08	22.9	35.1
General Average	20.87	61.79	29.37	2.76	10.03	5.35	40.68

*significant and ^{ns}no significant by the test F to 5%.

According to Pimentel-Gomes (2009), for field experiments with agricultural crops, CV values are low when they are lower than 10%, average when they are between 10 and 20%, high when they are between 20 and 30%, and very high, when they are higher than 30%.

The AUDPC characteristic had a mean value, showing that there was a good experimental precision, with mean CV, of 11.15% (Table 6). The mean values of AUDPC were 9.5 to 33, lower than those found by Moreira et al. (2015), from 12 to 45.8%, which also evaluated fruits of *C*. *annuum*. The high amplitude of AUDPC values is due to the evaluation of individuals from the segregating population, in which it was possible to identify plants with different levels of resistance to disease, as well as transgressive segregating genotypes.

The CVe of variation of FL and FD was 22.83 and 25.75%, respectively, being considered high. Bento (2012) evaluating fruits of *C. baccatum* also found high values, from 28.66 for FL and 20.83% for FD. Pimenta (2014) for the same characteristics in *C*. *annuum*, verified the CV of 11.53 (FL) and 4.87 (FD), considered medium and low. FL ranged from 31.05 to 111.86 mm and FD from 13.54 to 49.80 mm. Moreira et al. (2015) reported that FL varied between 39.07 and 112.02 mm and fruit diameter (FD) between 13.70 and 69.16 mm, values relatively close to those of this study. Domenico et al. (2012) evaluating agronomic characters in *C. chinense* observed fruit length between 2.1 and 7.7 cm and diameter of 1.1 to 2.5 cm in different accessions. Bhutia et al. (2015) evaluating genetic parameters in *C*. *annuum*, verified a mean of 3.49 to 8.8 cm for FL and 0.94 to 1.49 cm for PT.

The pulp thickness (PT) ranged from 1.28 to 11.95 mm and had a high CV of 21.95% (Table 6). The TSS presented values between 6.42 and 14.06 °Brix, among the evaluated characteristics, with CV of 10.08% (Table 6), the lowest among the evaluated variables. Pimenta (2014) found an average value similar to that of this experiment, of 10.2 °Brix. Close values were identified by Teodoro et al. (2013), ranging from 5 to 13 ºBrix, in *C. chinense* fruits. Bento (2012), found in *C*. *baccatum*, value of 6.40 to 13.40 °Brix.

Titratable acidity (TA) ranged from 1.28 to 11.28% citric acid (Table 6). Pimenta (2014) found average values of a maximum of 0.32, well below those of this study.

The VITc is a nutritional component and a natural antioxidant present in the fruits of *Capsicum*. Its concentration on fruits varies more and less according to genotype, maturity, management of fertilization and environmental factors (Bae et al., 2014). Despite the great importance of the vitamin C in human nutrition, it is still little commercially related to pepper fruits due to the low amount of pepper that

is normally consumed by a person (Frank et al., 2001). In this study, a maximum value of 83 mg $100g^{-1}$ was found. In the work of Pimenta (2014) values of vitamin C of 127 mg $100g^{-1}$ were verified. Bae et al. (2014), evaluating *C. annuum*, observed values between 1.95 and 137.3 mg $100g^{-1}$. Bhutia et al. (2015) found values of 83 mg $10g^{-1}$ to 211 mg $10g^{-1}$ in the fruits of *C. annuum*.

Regarding the estimated parameters, the features with the lowest environmental variance are AUDPC, PT, SSC and VITc (Table 6). This means that the variation of these characteristics is more related to the genetic factors. On the other hand, the characteristics FL, FD and TA had greater environmental influence.

The characteristic of bacterial stain resistance measured by AUDPC was the one with the highest heritability, with 82.41%, followed by PT, VITc and SSC. The FD, TA and FL characteristics were found to be median values of 49.20, 47.95 and 42.67, respectively (Table 7). Heritability is an essential parameter when it is desired that a certain characteristic be expressed in future generations, in which favorable alleles are passed on to the next generation.

In an early harvest of the F_2 population, it was possible to verify a production of the fruits, varying from 0.03 kg to 2.84 kg per plant. In fruits with pepper phenotype, the production was 3.0 kg $plant^{-1}$. Campos et al. (2008), evaluating the effect of nitrogen doses on the sweet pepper crop, obtained maximum production of 2.64 kg $plant^{-1}$. Oliveira et al. (2016), evaluating the efficiency of sweet pepper production under nitrogen and potassium fertirrigation, obtained a maximum production of 1.57 kg $plant^{-1}$. Both authors conducted the experiments in a protected environment. Thus, it can be affirmed that the sweet pepper production of this experiment was high, since it was conducted in experimental field.

The production of chilli pepper had value ranging from 0.06 to 1.1 kg $plant^{-1}$. This discrepancy in the weight of the peppers is due to the great variability found in the F_2 generation, in which small phenotypically fruits and other large were observed.

The selection was performed considering 30% in 188 of the F_2 progenies (Table 7), since the genetic gain above this percentage reduces each time the percentage of selection is increased. In the analysis of the Mulamba and Mock (1978) index, a higher evaluation weight was attributed to the AUDPC, FL and FD

characteristics, since the objective is to obtain a pepper resistant to the bacterial spot. For the other characteristics, the evaluative weight was lower than those previously mentioned. It was observed that for AUDPC the lower the value the better the control of the disease, because the smaller the area under the curve, the better the resistance of the plant to a given disease. The variable FF must also be considered, since according to *Bioversity International* (IPGRI), notes three and five are the pepper shapes.

Based on the Mulamba and Mock index (1978), the first presented promising values for obtaining genotypes with relevant characteristics of relevance. This index allows a combination of multiple characteristics, showing the possibility of a selection based on a single value, associating the others (Cruz and Carneiro, 2003).

The performance of the superior individuals selected in a plant breeding program should consider the agronomic characteristics and physical-chemical characteristics that add quality to the new varieties (Rêgo et al., 2009).

It was verified that five plants, individuals 152, 93, 126, 68 and 187 (in the order of the classification) were promising for the desired characteristics, making it possible to obtain a chili fruit resistant to bacterial spot, due to its characteristics of resistance, shape and absence of capsaicin (Table 7).

Most individuals selected by the index have capsaicin. Capsaicin among capsaicinoids is the most pungent and has antioxidant, anti-inflammatory and other properties (Wesolowska et al., 2011). Inheritance studies of capsaicinoids conclude that a gene determines pungency, that is, it is a monogenic inheritance trait and expression is associated with a dominant gene, *C*, on chromosome 2, necessary for the pungent genotypes to produce capsaicin. (Zewdie and Bosland, 2000; Blum et al., 2002).

The evaluated population is promising to obtain genetic gain by selection for most of the characteristics under study, since they present promising heritabilities, in particular for AUDPC, PT, SSC and VITc. However, for FL, FD and TA, with H^2 lower, difficulties in the genetic gain for these characters are considered.

Table 7. Plant selection by the selection index proposed by Mulamba and Mock (1978) for the agronomic characteristics and resistance to bacterial spot in generations of *C. annuum* var. *annuum*. UENF, Campos de Goytacazes, RJ, 2017.

Progeny F_2	Variables*								
	AUDPC	FL	FD	PT	SSC	TA	VITc	FF	CAPS
125	10.5	68.45	30.3	3.52	7.96	5.43	44.46	3	1
48	9.5	52.31	39.3	2	11.84	6.43	46.31	5	1
40	12.5	48.87	31.5	3.15	10.7	6.43	33.08	5	1
41	11.5	52.65	22.2	2.1	9.26	4.57	67.69	3	1
49	13	48.99	24.7	1.8	7.74	5.71	25.23	5	1
50	14	71.45	32.1	2.81	9.46	7.29	17.69	3	1
152	13.5	83.16	27.9	2.99	7.32	3.29	23.85	3	2
166	13.5	74.84	27.6	3.23	10.52	4.57	52	3	1
87	13.5	91.58	24.8	2.38	11.4	4.57	40.77	1	1
97	14.5	92.34	49.8	3.64	8.16	6.86	40	5	1
45	13.5	56.51	31.4	2.49	10.2	6	67.38	3	1
180	13.5	40.97	38.8	3.52	8.74	4.14	74.46	3	1
46	13.5	98.38	19.6	2.26	11.68	6.14	30.46	1	1
39	13.5	63.05	26.2	2.25	10.02	8.86	75.08	3	1
93	13.5	63.21	23.5	2.11	9.64	4.29	20.77	3	2
65	13.5	45.85	25.9	1.61	10.64	2.71	20.46	3	1
99	14.5	83.43	23.3	1.79	10.3	4.29	78.92	1	1
178	13.5	45.3	20.2	2.14	12.88	6.71	21.08	3	1
146	14.5	65.3	25.1	2.12	10.68	5.43	47.54	1	1
57	15	64.66	35.6	2.85	10.58	7.14	73.85	3	1
67	15	69.83	28.8	2.83	7.5	4.57	48.15	3	1
72	15	73.8	25.1	2.78	11.26	2.43	61.54	1	1
138	14.5	47.68	24.5	3.27	8.56	6.14	71.85	5	1
60	15	58.46	28.8	2.25	8.58	3.71	54.15	3	1
63	14.5	37.74	24.7	2.55	12.72	3.86	23.38	3	1
43	15	50.63	25.4	2.98	10.7	4	55.23	3	1
94	15.5	102.71	28.9	3.09	7.48	3.71	27.69	1	1
98	15.5	79.48	30.3	2.66	8.8	4.86	40	3	1
150	15.5	64.06	35.4	3.76	9.24	8.29	40.62	3	1
47	15.5	78.53	26.9	1.75	12.14	6.14	8.77	1	1
52	15.5	62.53	31.1	3.99	8.94	2.43	78.46	1	1
124	15.5	65.24	28.9	2.7	10.66	7	74.46	3	1
61	15.5	80.94	25.2	2.04	12.44	4.57	72	1	1
126	15.5	68.22	28	2.53	9.04	3.57	60	3	2
121	15.5	55.44	30.8	2.75	9.02	6	83.08	1	1
172	15.5	49.28	32.6	11.95	9.28	6.14	38.62	3	1
120	15.5	56.33	30	2.33	7.93	5.71	34.92	3	1
53	15.5	64.8	26.8	3	9.28	6.71	33.69	3	1
68	15.5	63.28	26.9	2.09	12.18	5.57	31.54	3	2

Table 7. Cont

Progeny F_2	Variables*								
	AUDPC	FL	FD	PT	SSC	TA	VITc	FF	CAPS
155	15.5	56.46	27.6	2.7	10.12	4	54.15	3	1
102	16	83.58	30.3	2.49	10.06	8	72.31	3	1
109	15.5	66.78	20.9	2.02	8.82	6.71	63.54	1	1
103	16.5	73.09	32.6	3.24	8.82	3.57	61.69	3	1
187	15.5	51.72	27.07	3.66	8.86	5.14	62.31	3	2
110	15.5	58.23	21.3	1.79	8	10.86	30.77	3	1
116	16	83.44	26.7	2.87	11.16	3.57	54.62	1	1
157	16	60.26	31.8	3.32	8.54	6.86	11.54	3	1
154	15.5	52.3	22.1	1.91	11.52	5.29	47.23	3	1
174	15.5	46.98	22	2.19	8.98	7.43	37.38	3	1
151	15.5	48.69	20.5	1.8	11.84	10.71	58.31	1	1
156	15.5	36.65	20.9	2.01	11.26	4.29	13.69	3	1
135	16	64.5	25.1	2.36	7.08	6.14	60.15	3	1
170	16	71.2	21.3	2.44	9.12	4.71	61.08	1	1
85	17	106.67	32.7	2.36	8.8	3.14	77.08	1	2
10	16	65.22	20.7	2.59	7.5	7.71	2.31	1	1
76	17	75.64	38.7	4.44	7.72	3.57	67.23	5	1
117	17	53.76	31.1	3.24	10.52	4.43	22.62	3	2
184	17	54.76	29.7	2.24	10.8	5.57	19.23	3	2
74	17	36.7	32.1	2.99	11.26	4	36.62	3	1
70	17	68.07	23.6	1.92	12.94	3.71	66.15	1	2

* Legend: AUDPC - area under the disease progress curve; FL - fruit length, FD - fruit diameter; PT - thickness of the pericarp; SSC - soluble solids content; TA - Titratable Acidity; VITc - vitamin C; FF - fruit format; CAPS - capsaicin.

5.4. New genes responsible for hypersensitive response in *Capsicum annuum* when inoculated with *Xanthomonas gardneri*

5.4.1. Identification of recessive resistance genes *bs5* and *bs6*

In Fig. 10.A, it can be verified that the *Bs4* gene is present only in tomato, by the presence of the band with 535bp. There was no amplification of bands associated with the *Bs4* genes in the UENF 2285 (susceptible) and UENF 1381 (resistant) bands (Figures 10 and 11). Therefore, the presence of the gene *Bs4* does not indicate that it is involved in the disease resistance observed in UENF 1381. As it can be observed in Figure 10.B, the gene *Bs4C* was only found in *C. pubescens*. *C. annuum* had amplicons for the gene *Bs4C*, but not an expected size band of 450bp.

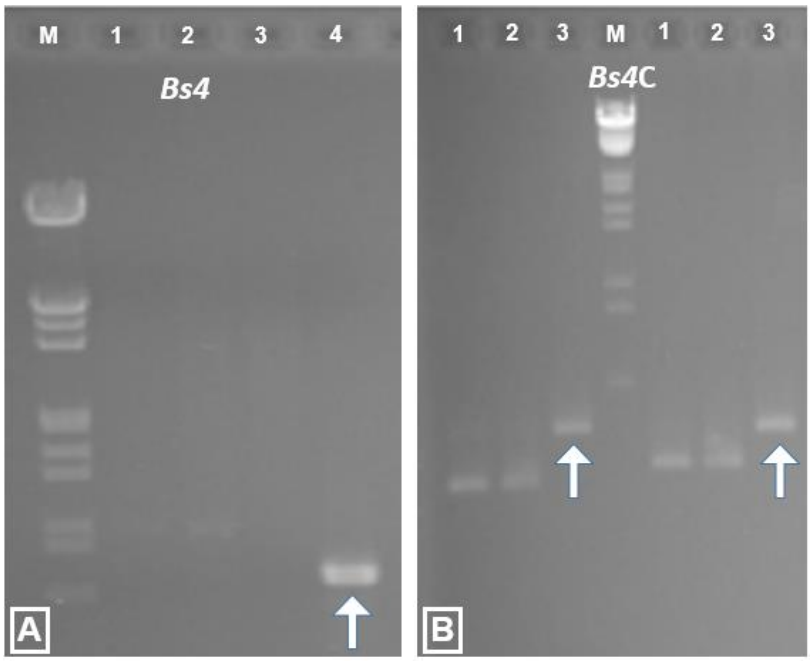

Figure 10. PRC gel for identifying the presence of the *Bs4* gene in *C. annuum*, *C. pubescens* and in tomato. (M - label, 1 – UENF 1381, 2 – UENF 2285, 3 - *C. pubescens* 235047 and 4 - Bonny Best Tomato)

In Figure 6, the PCR product displays a differentiated band pattern for the parents and the hybrids, regarding the samples containing the different recessive genes. Thus, the different band pattern in the gel (samples number 4 and 5) shows that the *C. annuum* population in this study seems not to carry the genes *bs5* and *bs6*. Howeve, the expected band size information is not available in the literature.

In 2004, Riva et al. carried out the study of inheritance of resistance to bacterial blight in a population of *C*. *annuum*, using UENF 1381 as a source of resistance, and identified the presence of three recessive genes responsible for resistance control.

Thus, in the absence of recessive genes *bs5* and *bs6*, a series of inoculations with several species and races of *Xanthomonas* was started to better understand the resistance response in UENF 1381.

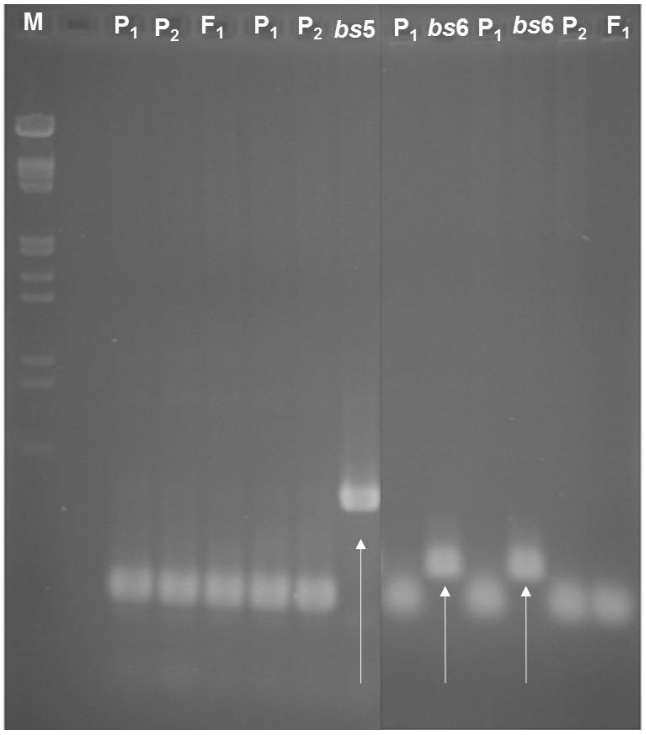

Figure 11. PCR gel for identification of the presence of genes *bs5* and *bs6* in *C. annuum* (M - label, P_1 – UENF 1381, P_2 – UENF 2285, F_1 – UENF 2285 x UENF 2285, *bs5* and *bs6*).

In the HR tests, in the access UENF1381 against *avrBs1*, *avrBs2* and *avrBs3* using different races *X. euvesicatoria* and *X. gardneri,* it was identified that the plants of this access do not possess these genes. Molecular identification was not performed because there are no PCR primers to detect resistance genes in pepper.

5.4.2.Resistance of UENF 1381 to different isolates of *Xanthomonas* spp.

The bacterial population, with the four species of *Xanthomonas*, presented differentiated growth among the parents of *Capsicum*. The female genitor, UENF 2285, susceptibility to bacterial spotting pattern, presented for all species of *Xanthomonas* formation of bacterial colonies larger than UENF 1381.

During the 10 days of evaluation, UENF 2285 showed a gradual formation of colonies (Figure 12). This result is expected because this access is a standard of susceptibility. Susceptibility results of this genotype were identified by Stall et al. (2009), Potnis et al. (2011) and Silva et al. (2017) in works with similar pipeline.

UENF 1381, inoculated with *X. euvesicatoria*, presented a reduction in colony formation on the sixth day of evaluation. The same result can be visualized when this access was inoculated with *X. gardneri* and *X. perforans*. The responses of this parent confirm that the defense mechanisms are active, delaying the penetration of the pathogens and creating conditions that are inappropriate for the development of *Xanthomonas* that were inoculated in the leaf tissue (Agnelli, 2011). UENF 1381 expression expresses non-specific resistance through the activation of genes involved in defense responses (Lamb and Dixon, 1997).

Vallejos et al. (2010) affirmed that there are genes that are effective, alone or in a joint action, to present resistance to bacterial spotting shortly after infection. Riva et al. (2004), Riva-Souza et al. (2009), Moreira et al. (2015), Pimenta et al. (2016) and Silva et al. (2017) also observed resistance of UENF 1381 to *Xanthomonas* in their research.

The largest difference observed in the bacterial population between the accessions was with the infiltration of *X. euvesicatoria* (ENA 4135) and *X. gardneri* (Figure 12). These results showed that the virulence of the pathogen acted as expected in the parents, since they are considered as contrasting for the characteristic of bacterial stain resistance.

The plants were inoculated with *X. cynarae*, but they did not present a reaction to this bacterium, since they are not pathogenic in *C. annuum*.

The results of the quantitative evaluation of *X. euvesicatoria* (ENA 4135) and *X. gardneri* (444) showed that in the parental and generation F_1 the expected proportion for these bacterial species occurred (Table 8). In the parental UENF 2285 (P_1), susceptible, the ratio was zero resistant to three susceptible and in the resistant parental, UENF1381 (P_2), six resistant to zero susceptible. All F_1 plants were susceptible.

The concentration of the inoculum used in this assay allowed the identification of resistant and susceptible F_2 individuals by note scale through observation the appearance of pustules in the abaxial part of the inoculated leaf.

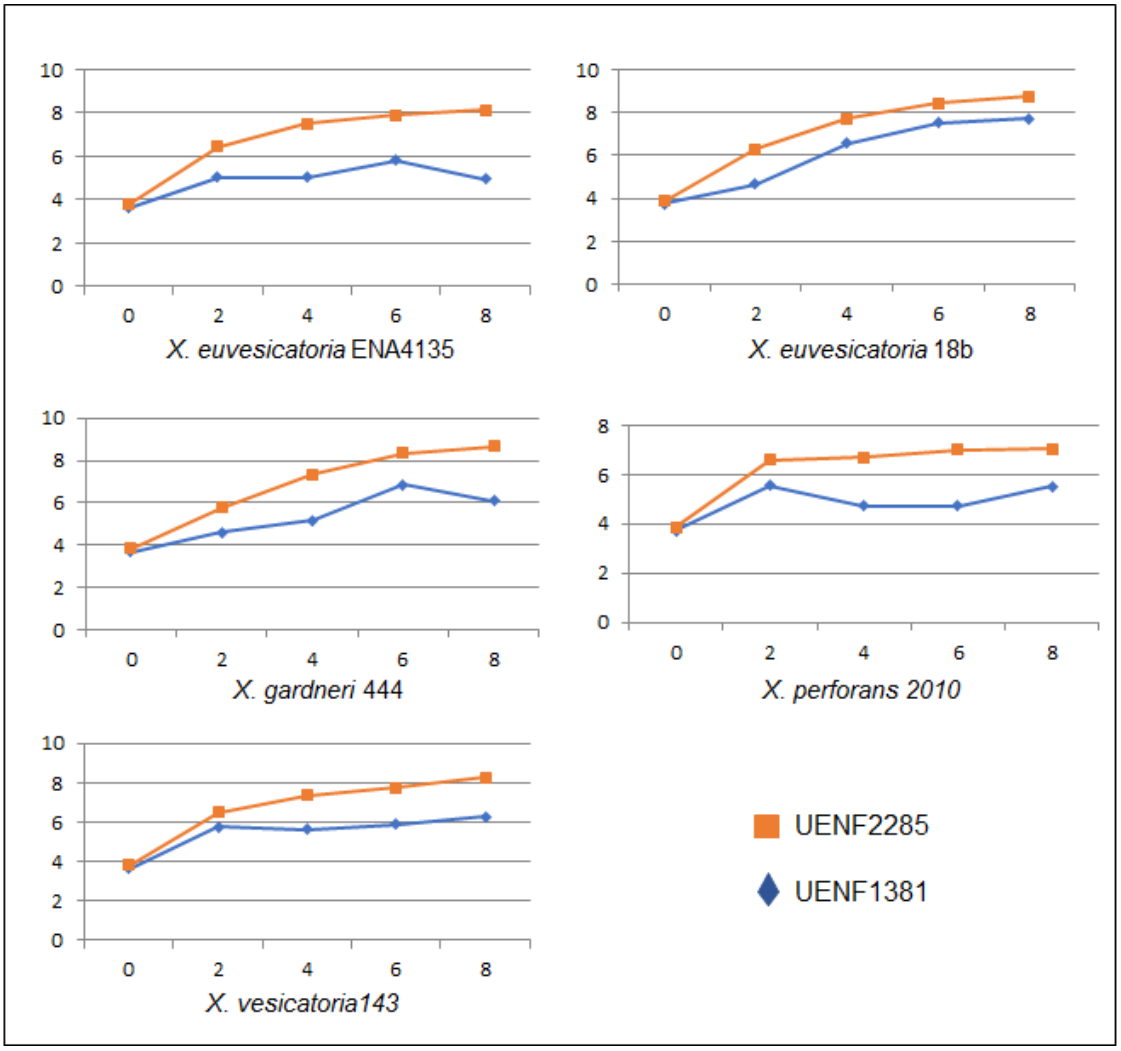

Figure 12. Virulence of different species of *Xanthomonas* in both parents, UENF 1381 and UENF 2285, of *C. annuum* var. *annuum*, during the course of eight evaluations.

It was verified by the chi-square test that for the race of *X. euvesicatoria* (ENA4135) there is a probability (70.79%) of two genes related to the resistance, already for the race 444 of *X. gardneri* the probability is almost 100% of two genes associated with resistance (P≤0.05).

It was also observed in the used controls that the almost isogenic lines ECW50R and ECW60R were resistant to the *X. euvesicatoria* race and susceptible to the *X. gardneri* breed (Table 8).

Table 8. Reaction of resistance to bacterial spot in generations of *C. annuum* var. *annuum* inoculated with a race of *X*. *euvesicatoria* (ENA 4135) and *X*. *gardneri* (444).

G.	***X. euvesicatoria* (ENA4135)**							G.	***X. gardneri* (444)**						
	O		E		hypothesis	χ^2	(*P*%)		O		E		hypothesis	χ^2	(*P*%)
	R	S	R	S					R	S	R	S			
P_1	0	3	0	3	-	-	-	P_1	0	3	0	3	-	-	-
P_2	6	0	6	0	-	-	-	P_2	6	0	6	0	-	-	-
F_1	0	6	0	6				F_1	0	6	0	6			
F_2	25	35	15	45	**9:7** (2 genes)	0.1403*	70.79	F_2	3	57	3.75	56.25	**15:1** (2 genes)	0.0008*	97.61
Controls								Controls							
ECW50R	3	0	3	0	-	-	-	ECW50R	0	3	0	3	-	-	-
ECW60R	3	0	3	0	-	-	-	ECW60R	0	3	0	3	-	-	-

Concentration of inoculation $2x10^3$; G. – generation; (*P*) – probabilitiy; χ^2 (1DF) 5% = 3.84

The hypothesis that resistance to *X. campestris* pv *campestris* is attributed by the action of three genes fits the ratio of 51:13, with a low probability (37.46%), but significant (P≤0.05) by the chi-square (χ^2: 0.78) (Table 9).

Table 9. Evaluation of resistance to bacterial spot in generations of *C. annuum* var. *annuum* inoculated with a race of the *X. campestris* pv*. campestris.*

Generations	***X. campestris* (R10)**						
	O		E		Hypothesis	χ^2	(*P%*)
	R	S	R	S			
P_1	6	0	6	0	-	-	-
P_2	6	0	6	0	-	-	-
F_2	16	80	19.6	76.5	**51:13** (3 genes)	0.78*	37.46

Concentration of inoculation $2x10^5$; (*P*) – probability; χ^2 (1FD) 5% = 3.84

In this study, it is possible to verify by chi-square (χ^2: 0.0745; P=78.48%) that the characteristic evaluated in this population is controlled by three genes (P≤0.05), confirming that it is an oligogenic inheritance disease (Table 10). It is also verified that in both isolates of *X. campestris* the parental UENF 1381 was susceptible. This is because *X. campestris* pv. *campestris* is specific to the brassicas and in *X. campestris* pv. *vesicatoria* the access UENF 1381 has its has non-specific race resistance (quantitative resistance) as can be observed in table 13.

Table 10. Hypersensitivity reaction to bacterial spot in generations of *C. annuum* var. *annuum* inoculated with a race *X. euvesicatoria* and *X. campestris* pv *campestris*.

G.	***X. euvesicatoria***				G.	***X. campestris* pv. *campestris***			
	O		E			O		E	
	HR	S	HR	S		HR	S	HR	S
P_1	0	6	-	-	P_1	0	6	-	-
P_2	0	6	-	-	P_2	0	6	-	-
F_2	0	96	-	-	F_2	0	96	-	-

Concentration of inoculation $2x10^8$; G – generation; (*P*) – probability; χ^2 (1FD) 5% = 3.84

In Table 11, for the 51 breed of *X. gardneri*, despite the low probability (55.34%), it suggests two genes responsible for the resistance, significant at 5%. For the 444 race, there are almost 100% probability of being three genes.

After verifying these quantitative results for *X. gardneri*, with potential for resistance, the plants were inoculated with two isolates of *X. gardneri* (51 and 444), to evaluate the HR (Table 11).

Table 11. Hypersensitivity reaction to bacterial spot in generations of *C. annuum* var. *annuum* inoculated with two races of *X*. *gardneri* (race 51 and 444).

G.	***X. gardneri* 51**							G.	***X. gardneri* (444)**						
	O		E		Hypothesis 1	χ^2	(*P%*)		O		E		Hypothesis 2	χ^2	(*P%*)
	HR	S	HR	S					HR	S	HR	S			
P_1	0	3	0	3	-	-	-	P_1	0	3	0	3	-	-	-
P_2	6	0	6	0	-	-	-	P_2	6	0	6	0	-	-	-
F_1	6	0	6	0	-	-	-	F_1	0	6	0	6	-	-	-
F_2	56	4	56.25	3.75	**15:1** (2 genes)	0.35*	55.34	F_2	54	6	53.44	6.56	**57:7** (3 genes)	0.001*	96.53

Concentration of inoculation $2x10^8$; G. – generation; (*P*) – probability; χ^2 (1FD) 5% = 3.84

It has also been estimated that resistance to *X. gardneri* 51 is also controlled by three genes. The probability for this hypothesis is low, from 37, 22%, significant at 5%. The best hypothesis was that three genes control the hypersensitivity reaction, with 78.48% probability (Table 12).

Table 13 shows that for the race ENA 4135 the hypothesis that three genes is responsible for the resistance is best explained in hypothesis 1, with 87.01% probability. The probability that there are three genes in the control of resistance to *X. euvesicatoria* 0143 is low, but among the models tested, it was significant (P≤0.05). According to estimates of chi-square (0.02), there was a significance (P≤0.05) for the hypothesis of three genes responsible for resistance to *X. euvesicatoria 18b* in the quantitative evaluation (Table 13).

The results observed corroborate the results found by Riva et al. (2004) who, using the same evaluation method, stated that resistance to bacterial blight in a *C. annuum* population from a cross with UENF 1381 is polygenic, estimating the minimum number of three recessive genes.

Table 12. Hypersensitivity reaction to bacterial spot in generations of *C. annuum* var. *annuum* inoculated in the race 51, of *X. gardneri.*

	***X. gardneri* 51**													
G.	O		E		Hypothesis 1	χ^2	(*P%*)	G.	O		E		Hypothesis 2	χ^2	(*P%*)
	HR	S	HR	S					HR	S	HR	S			
P_1	0	6	-	-	-	-		P_1	0	6	-	-	-	-	-
P_2	6	0	-	-	-	-		P_2	6	0	-	-	-	-	-
F_2	61	35	73.5	22.5	**43:21** (3 genes)	0.796*	37.22	F_2	42	54	34.68	25.31	**37:27** (3 genes)	0.07*	78.48

Concentration of inoculation $2x10^8$; G. – generation; (*P*) – probability; χ^2 (1FD) 5% = 3.84

Table 13. Scale of grades in *C. annuum* var. *annuum* inoculated with a race *X. euvesicatoria* ENA 4135, *X. euvesicatoria* 18b and *X. eucesicatoria* 0143.

	***X. euvesicatoria* ENA 4135**													
G.	O		E		Hypothesis 1	χ^2	(*P%*)	G.	O		E		Hypothesis 2	χ^2	(*P%*)
	R	S	R	S					R	S	R	S			
P_1	0	6	0	6	-			P_1	0	6	0	6	-	-	-
P_2	6	0	6	0	-			P_2	6	0	6	0		-	-
F_2	86	10	85.5	10.5	**57.7** (3 genes)	0.026*	87.01	F_2	80	16	76.5	19.5	**51:13** (3 genes)	0.78*	37.46

	***X. euvesicatoria* 0143**							***X. euvesicatoria* 18b**							
G.	O		E		Hypothesis	χ^2	(*P%*)	G.	O		E		Hypothesis	χ^2	(*P%*)
	R	S	R	S					R	S	R	S			
P_1	0	6	0	6	-	-	-	P_1	0	6	0	6	-	-	-
P_2	6	0	6	0		-	-	P_2	6	0	6	0	-	-	-
F_2	48	48	55.5	40.5	37:27 (3 genes)	0.99*	31.97	F_2	10	86	10.5	85.5	57:7 (3 genes)	0.02*	87.01

Concentration of inoculation $2x10^5$; (*P*) – probability; χ^2 (1FD) 5% = 3.84

An F_2 plant with promising results for resistance to different species of *Xanthomonas* was observed during the inoculations. Thus, a preliminary test using a few plants was carried out in which different races of *X. gardneri* were inoculated. Three plants of UENF 2285 and of the hybrid were evaluated, four of UENF 1381 and plant #6 (F_2). All the plants of UENF 1381 and #6 presented strong HR to all species (Table 14).

The UENF 1381 accession has shown relevance for studies of bacterial stain resistance. Thus, Different races of each species of Xanthomonas were inoculated in this access to verify hypersensitivity reaction (Table 14).

Table 14. Hypersensitivity reaction to different races of *X. euvesicatoria*, *X. gardneri, X. perforans, X. vesicatoria*, in accession of *C. annuum*, UENF 1381.

Xanthomonas	**UENF 1381**
***X. euvesicatoria*:**	
P3 (ENA 4135 Brazil)	S*
T1P10 (e18b Florida)	S
P1 (BA26-1 Argentina pepper)	HR
***X. gardneri*:**	
444 (Costa Rica)	HR
51 (Canadá)	HR
10 (Embrapa Hortaliças)	S
1782 (Brazil)	HR
1783 (Brazil)	HR
***X. perforans*:**	
2010 (Florida pepper)	I
RR110 (Australia tomato)	I
***X. vesicatoria*:**	
T2P3 BA29-1 (Argentina 143 tomato)	S
T2P3 BA21-4 (Argentina Pepper 607)	S

* S - susceptible, HR - hypersensitivity reaction, I - intermediate.

Inoculation with the P3 and T1P10 races of *X. euvesicatoria* did not result in HR. These results were already expected and confirm the work of Silva et al. (2017), who also used these races in studies of resistance evaluation and there was no HR reaction in *C. annuum* plants.

The five races of *X. gardneri* produced a very characteristic HR, however, for *X. perforans* this HR was intermediate, whereas there was no HR record for any *X. vesicatoria* race.

The UENF 1381 access was resistant to three of the five races of X. vesicatoria and plant # 6 (F_2) presented HR to two of them (Table 15). Both were resistant to the RR110-AUS14 race of *X. perforans*. These results show that the accession UENF 1381 as well as the plant # 6 are promising for the breeding to *Xanthomonas* spp. resistance.

Recent studies show that the evolution of this group of bacteria is constant. Over the years, researchers note that more and more varieties of new virulence vectors appear (Potnis et al., 2015). The spread of these bacteria throughout the world is increasingly intense (Timilsina et al., 2014). Therefore, it reinforces the importance in obtaining genotypes with resistance to *Xanthomonas*.

Table 15. Evaluation of hypersensitivity reaction in different races of *X. vesicatoria* (from 1 to 5) and *X. perforans* (6) in an individual of F_2 (plant 6) and in UENF 1381.

Race	**Plant F_2 (# 6)**	**UENF 1381**
1. BA29-1-143	*S	S
2. BA26-1-611	S	HR
3. BA26-4-620	HR	HR
4. BA21-1-606	HR	HR
5. BA21-4-607	S	S
6. RR110-AUS14	HR	HR

*S – susceptible, HR – hypersensitivity reaction.

6. CONCLUSÕES

Five genes, predominantly recessive, control resistance to bacterial spot in populations derived from the crossing between accessions UENF 2285 and UENF 1381.

Genetic control of bacterial spot has a quantitative aspect, with higher additive effect. Therefore, it is recommended to use breeding methods that allow selection of most advanced generations when the traits are already fixed, thus reducing the environmental effects.

The inheritance of resistance to bacterial blight in generations of *C*. *annuum* var. *annuum* from the cross between UENF 2285 x UENF 1381 is polygenic, with a larger gene with additive effect associated with polygenes with additive and dominance effect, being a disease of a complex genetic nature.

It was possible to select 35 promising genotypes to obtain a bacterial blight resistant pepper and also have nutritional properties that please the consumer market of this product.

The variation of the characteristics of resistance to bacterial spotting (AUDPC), PT, SSC and VITc are associated more to genetic than environmental factors, which facilitates their fixation in breeding programs.

The genes *Bs4*, *Bs4C*, *bs5* and *bs6* are not present in the parent UENF 1381 and consequently, neither in the population from crosses between UENF 2285 x UENF 1381. We can suggest that, in this case, possible new genes are

influencing hypersensitivity reactions observed in the control of resistance to bacterial spot.

UENF 1381 accession is a very valuable genetic resource for bacterial spot resistance breeding programs in *Capsicum*, since it has quantitative and qualitative resistance to the different species of *Xanthomonas*.

BIBLIOGRAPHIC REFERENCES

Abreu, F. B., Silva, D. J. H., Cruz, C. D., Mizubuti, E. S. G. (2008) Inheritance of resistance to *Phytophthora infestans* (*Peronosporales*, *Pythiaceae*) in a new source of resistance in tomato (*Solanum* sp., *Lycopersicon* sp.), (Solanaceae). *Genetic and Molecular Biology,* v. 31, p. 493-497.

Agnelli, A. R. (2011) Potencial de agentes indutores de resistência para o controle da bactéria *Candidatus* Liberibacter asiaticus em plantas cítricas. Dissertação (Mestrado) Araraquara, p. 44

Aguiar, L. A., Kimura, O., Castilho, A. M. C., Castilho, K. S. C. (2000) Resistência ao cobre em isolados nacionais de *Xanthomonas campestris* pv. *vesicatoria* de pimentão e tomateiro. *Agronomia,* v. 34, p. 78-82.

Amaral, A. L., Carvalho, F. I. F., Federizzi, L. C., Mittelmann, A. (1996) Estimativa de herdabilidade para os caracteres adaptativos ciclo e estatura de planta em aveia. *Ciência Rural,* v. 26, p. 33-37.

Andrews, J. (1995) Peppers: the domesticated *Capsicum*. Austin: *University of Texas* Press, p. 170.

Associação Brasileira de Saúde Coletiva - Dossiê ABRASCO (2015). *Um alerta sobre os impactos dos agrotóxicos na saúde*. Parte 1 - Agrotóxicos, Segurança Alimentar e Nutricional e Saúde.

Bae, H., G.K. Jayaprakasha, K. Crosby, K.S. Yoo, D.I. Leskovar, J. Jifon, and B.S. Patil. (2014). Ascorbic acid, capsaicinoid, and flavonoid aglycone concentrations as a function of fruit maturity stage in greenhouse-grown peppers. *Journal of Food Composition and Analysis*, v. 33, n. 2, p. 195-202.

Baldissera, J. N. C., Valentini, G., Coan, M. M. D., Guidolin, A.F., Coimbra, J. L. M. (2014) Fatores genéticos relacionados com a herança em populações de plantas autógamas. *Revista de Ciências Agroveterinárias,* v. 13, n.2, p. 181-189.

Barbosa, G.E., Agra, M.F., Romero, M.V., Scaldaferro, M.A., Moscone, E.A. (2011) New endemic species of *Capsicum* (Solanaceae) from the Brazilian Caatinga: comparison with the re-circumscribed *C. parvifolium*. *Systematic Botany*, v. 36, p. 768-781.

Barbosa, G.E. (2011) Lectotypifications, synonymy, and a new name in *Capsicum* (Solanoideae, Solanaceae). *Phytokeys*, v. 2, p. 23-38.

Barroca, M. V., Bonomo, R., Fernandes A. A., Souza, J. M. (2015) Lâminas de irrigação nos componentes de produção das pimentas “De cheiro” e ‘Dedo-de-Moça. *Revista Agro ambiente On-line*, v. 9, n. 3, p. 243.

Batista, R. O., Silva, L. C., Moura, L. M., Souza, M. H., Carneiro, P. C. S., Carvalho Filho, J. L. S., Carneiro, J. E. S. (2017) Inheritance of resistance to *fusarium* wilt in common bean. *Euphytica*, p. 213-133.

Bento, C. S., Souza, A. G., Sudré, C. P., Pimenta, S., Rodrigues, R. (2017) Multiple genetic resistances in *Capsicum* spp. *Genetic and Molecular Research,* v. 16, n.3.

Bento, C. S., Rodrigues, R., Gonçalves, L. S. A., Oliveira, H. S., Santos, M. H., Pontes, M. C., Sudré, C.P. (2013) Inheritance of resistance to Pepper yellow mosaic virus in *Capsicum baccatum* var. *pendulum*. *Genetic and Molecular Research,* v. 12, n. 2, p. 1074-1082.

Bento, C. S. (2012) Melhoramento de *Capsicum baccatum* var. *pendulum*: herança de caracteres agronômicos e resistência ao *Pepper yellow mosaic*

virus. Tese (doutorado) – Campos dos Goytacazes – RJ, Universidade Estadual do Norte Fluminense – UENF, 159p

Bergamin Filho, A., Amorim, L. (2002) Doenças com período de incubação variável em função da fenologia do hospedeiro. *Fitopatologia brasileira,* v. 27, p. 561-565.

Bhutia, N. D.; Seth, T.; Shende, V. D.; Dutta, S.; Chattopadhyay, A. (2015). Estimation of Heterosis, dominance effect and genetic control of fresh fruit yield, quality and leaf curl disease severity traits of chilli pepper (*Capsicum annuum* L.). *Scientia Horticulturae*, v. 182, p. 47–55.

Bianchetti, L., Carvalho, S. I. C. (2005) Subsídios à coleta de germoplasma de pimentas e pimentões do gênero *Capsicum* (Solanaceae). In: Walter, B. M. T., Cavalcanti, T. B. *Fundamentos para coleta de germoplasma vegetal.* Brasília: Embrapa Recursos Genéticos *e* Biotecnologia, p. 355-385.

Bongiolo Neto, A., Reifschneider, F. J. B., Takatsu, A. (1986) Levantamento de grupos de *Xanthomonas campestris* pv. *vesicatoria* no Brasil. *Fitopatologia Brasileira*, v.11, n. 2, p.881-889.

Bosland, P. W. (1996) *Capsicum*: innovative uses of an ancient crop. In: JANICK, J. (Ed.). *Progress in new crops*. Arlington: ASHS Press, p. 479-487.

Blum, E., Liu, K., Mazourek, M., Yoo, E. Y., Jahn, M., Paran, I. (2002) Molecular mapping of the C locus for presence of pungency in *Capsicum*. *Nature*, v. 45, n. 4, p. 702 – 705.

Campos, V. B., Oliveira, A. P., Cavalcante, L. F., Prazeres, S. S. (2008) Rendimento do pimentão submetido ao nitrogênio aplicado via água de irrigação em ambiente protegido. *Revista de Biologia e Ciências da Terra*, v. 8, n. 2.

Carias, C. M. de O. M.; Gravina, G. do A.; Ferrão, M. A. G.; Fonseca, A. F. A. da; Ferrão, R. G.; Vivas, M.; Pio Viana, A. (2016) Predição de ganhos genéticos via modelos mistos em progênies de café conilon. *Coffee Science*, v.11, n.1, p.39-45.

Carmo, M. G. F., Zerbini Júnior, F. M., Maffia, L. A. (2006) Principais doenças da cultura da pimenta. *Informe Agropecuário*, Belo Horizonte, v. 27, n.235, p. 87-98.

Carvalho, S. I. C, Bianchetti, L. B. (2008) *Botânica e recursos genéticos.* In: Ribeiro, C. S. C., Lopes, C. A., Carvalho, S. I. C., Henz, G. P., Reifschneider, F. J. B. Pimentas *Capsicum*. Brasília: *Embrapa Hortaliças*, p. 39–53.

Carvalho, H. H., Wiest, J. M., Cruz, F. T. (2010) Atividade antibacteriana in vitro de pimentas e pimentões (*Capsicum* sp.) sobre quatro bactérias toxinfectivas alimentares. *Revista Brasileira de Plantas Medicinais*, Botucatu, v. 12, n. 1, p. 8-12.

Castellani, A. (1939) Viability of some pathogenic fungi in distilled water*. American Journal of Tropical Medicine and Hygiene*, v. 42, p. 225.

Castle, W. E. (1921) An improved method of estimating the number of genetic factors concerned in cases of blending inheritance. *Science*, v. 54, p. 223.

Chiou, K. L., Hastorf, C. A. A. (2014) Systematic Approach to Species–Level Identification of Chile Pepper (*Capsicum* spp.) Seeds: Establishing the Groundwork for Tracking the Domestication and Movement of Chile Peppers through the Americas and Beyond. *Economic Botany*, setembro, v. 68, n. 3, p. 316–336.

Cook, A. A., Stall, R. E. (1982) Distribution of races of *Xanthomonas vesicatoria* pathogenic on pepper. *Plant Disease*, v. 66, n. 5, p. 388-389.

Costa, R. A., Rodrigues, R., Sudré, C. P. (2002) Resistência genética à mancha bacteriana em genótipos de pimentão. *Horticultura brasileira,* v. 20, n. 1, p. 86-89.

Costa, C. S. R., Henz, G. P. (2007) *Pimenta* (*Capsicum* spp.). Embrapa Hortaliças.

Cruz, C. D., Regazzi, A. J., Carneiro, P. C. S. (2014) *Modelos biométricos aplicados ao melhoramento genético*. Viçosa-MG. Ed. UFV, v. 2, 3° ed., p. 14.

Cruz, C. D. (2013) GENES - A Software Package for Analysis in Experimental Statistics and Quantitative Genetics. *Acta Scientarum Agronomy*, v. 35, p. 271-276.

Cruz, C. D., Regazzi, A. J., Carneiro, P. C. S. (2012) *Modelos biométricos aplicados ao melhoramento genético*. Editora UFV, Viçosa, v. 1, 4 ed., p. 514.

Cruz, C.D., Carneiro, P.C.S. (2003) Modelos biométricos aplicados ao melhoramento de plantas. v.2. Viçosa: UFV, 585p.

Custódio, D. L., Burgo, R. P., Moriel, B., Barbosa, A. M., Rezende, M. I., Daniel, J. F. d. S., Pinto, J. P., Bianchini, E., Faria, T. J. (2010) Antimicrobial activity of essential oils from Pimenta *pseudocaryophyllus* and *Tynanthus micranthus*. *Brazilian Archives of Biology and Technology*, v. 53, p. 1363-1369.

Demosthenes, L. C. R., Bentes, J. L. S. (2011) Fontes de resistência à murcha bacteriana em germoplasma de *Capsicum* spp. do estado do Amazonas. *Acta Amazônica,* v. 41, n. 3, p. 435-438.

Dias, G. B., Gomes, V. M., Moraes, T. M., Zottich, U. P. (2013) Characterization of *Capsicum* species using anatomical and molecular data. *Genetics Molecular Research,* v. 12, p. 6488-6501.

Diniz, G. M. M. (2016) Controle genético da resistência de *Capsicum frutescens* à *Meloidogyne enterolobii*. Tese (doutorado) – Universidade Estadual Paulista, Faculdade de Ciências Agrárias, xi, p. 53.

Embrapa Hortaliças. (2007) *Sistemas de Produção, 2*. ISSN 1678-880x, Versão Eletrônica.

FAO. *Food and Agriculture Organization of the United Nations.* Disponível em: . Acesso em: 17 de janeiro de 2017.

FAOSTAT (*Food and Agriculture Organization of the United Nations - Statistics Division*). Production/crops: Chillies and peppers. Available in: http://faostat3.fao.org/browse/Q/QC/E. Acessado em: 10/12/2016.

Ferreira, M. G. (2017) Herança da resistência de *Solanum habrochaites* a *Phytophthora infestans* (Mont.) De Bary. Dissertação (Mestrado) – Universidade Federal de Viçosa, vii, 27f.

Filgueira, F. A. R. (2012) *Novo Manual de Olericultura: Agrotecnologia Moderna na Produção e Comercialização de Hortaliças*. Editora UFV, 3° ed.

Fisher, D. S. (1918) The correlation between relatives on the supposition of Mendelian inheritance. *Transactions of the Royal Society of Edinburgh*, v. 52, p. 399-433.

Frank, C. A., Nelson, R. G., Simonne, E. H., Behe, B. K., Simonne, A. H. (2001) Consumer preferences for color, price, and vitamin C content of bell peppers. *HortScience*, v. 36, p. 795-800.

Freitas, I. L. J., Pio Viana, A., Pena, G. F., Cabral, P. S., Vittorazzi, C., Silva, T. R. C. (2013) Ganho genético avaliado com índices de seleção e com REML/Blup em milho-pipoca. *Pesquisa agropecuária brasileira*, Brasília, v.48, n.11, p.1464-1471.

Fulton, T. M., Chunzoongse, J., Tanksley, S. D. (1995) Microprep Protocol for Extraction of DNA from Tomato and other Herbaceous Plants*. Plant Molecular Biology,* v. 13, n.3, p. 207-209

García, C. C., Barfuss, M. H., Sehr, E. M., Barboza, G. E., Samuel, R., Moscone, E. A., Ehrendorfer, F. (2016) Phylogenetic relationships, diversification and expansion of chili peppers (*Capsicum*, Solanaceae). *Annals of Botany*, julho, v. 118, n. 1, p. 35–51.

Gonçalves, P. de S., Bortoletto, N., Martins, A. L. M., Costa, R. B. da, Gallo, P. B. (2003) Genotype-environment interaction and phenotypic stability for girth growth and rubber yield of Hevea clones in São Paulo State, Brazil. *Genetics and Molecular Biology*, Ribeirão Preto - SP, v. 26, n. 4, p. 441-448.

Hamza, A. A., Robène-Soustrade, I., Jouen, E., Gagnevin, L., Lefeuvre, P., Chiroleu, F., Pruvost, O. (2010) Genetic and pathological diversity among *Xanthomonas* strains responsible for bacterial spot on tomato and pepper in the southwest Indian Ocean region. *Plant Disease*, v. 94, p. 993-999.

Hazel, L. N. (1943) The genetic basis for constructing selection indexes. *Genetics*, Austin, v. 28, p. 476-490.

Hu, Y., Chen, L., Wang, H., Zhang, L., Wang, F., Yu, D. (2013) Arabidopsis transcription factor WRKY8 functions antagonistically with its interacting partner VQ9 to modulate salinity stress tolerance. *The Plant Journal*, v. 74, p. 730–745.

Ince, A. G., Karaca, M., Onus, A. N. (2010) Genetic relationships within and between *Capsicum* species. *Biochemical Genetics*, v. 48, p. 83-95.

IPGRI (1995) Descriptors for *Capsicum* (*Capsicum* spp.) International Plant Genetic Resources Institute, p. 49.

Jones, J. B, Stall, R. E. (1998) Diversity among Xanthomonads pathogenics on pepper and tomato. *Annual Review of Phytopathology*, v. 36, p. 41-58.

Jones, J. B., Minsavage, G. V., Roberts, P. D., Johnson, R. R., Kousik, C. S., Subramanian, S., Stall, R. E. (2002) A non-hypersensitive in pepper to the bacterial spot pathogen is associated with two recessive genes. *Phytopathology*, v. 92, p. 273-277.

Jones, J.B., Lacy, G. H., Bouzar, H. Stall, R. E., Schaad, N. W.. (2004) Reclassification of the Xanthomonads associated with bacterial spot disease of tomato and pepper. *Systematic Applied Microbiology*, 27:755-762.

Juhász, A. C. P., Silva, D. J. H., Zerbini Júnior, F. M., Carneiro, P. C. S., Soares, B. O., Cruz, C. D. (2008) Base genética da resistência de um acesso de tomate silvestre ao mosaico-amarelo do pimentão. *Pesquisa agropecuária brasileira,* v. 43, n. 6, p. 713-720.

Juhász, A. C. P. (2002) Herança da resistência à mancha bacteriana em pimentão e avaliações estruturais associadas à interação *Xanthomonas axonopodis* pv. *vesicatoria* – *Capsicum annuum* L. Dissertação (Mestrado) – Campos dos Goytacazes – RJ, Universidade Estadual do Norte Fluminense – UENF, 86p

Kim, S., Park, M., Yeom, S. I., Kim, Y. M., Lee, J. M., Lee, H. A., Seo, E., Choi, J., Cheong, K., Kim, K. T., Jung, K. (2014) Genome sequence of the hot

pepper provides insights into the evolution of pungency in *Capsicum* species. *Nature Genetics*, março, v. 46, n. 3, p. 270–278.

Kurozawa, C., Pavan, M. A. (2005) Doenças do tomateiro (*Lycopersicon esculentum*). In: Kimati, H., Amorim, L., Rezende, J. A. M., Bergamin Filho, A., Camargo, L. E. A. Manual de fitopatologia: doenças das plantas cultivadas. 4ª ed. São Paulo: *Agronômica Ceres*, v.2, n. 8, p.607-626.

Lamb, C., Dixon, R. A. (1997) The oxidative burst in plant disease resistance. *Annual Review of Plant Physiology and Plant Molecular Biology*, v. 48, p. 251-275.

Leite, W. S., Pavan, B. E., Matos Filho, C. H. A., Alcântara Neto, F., Oliveira, C. B., Feitosa, F. S. (2016) Estimativas de parâmetros genéticos, correlações e índices de seleção para seis caracteres agronômicos em linhagens F_8 de soja. *Comunicata Scientiae*, v. 7, n.3, p. 302-310.

Lim, T. K. (2013) Edible medicinal and non-medicinal plants *Capsicum baccatum* var. *pendulum.Plant Sciences,* v.6, cap. 23, p. 202.

Lobo, V. L. S, Giordano, L. B., Lopes, C. A. (2005) Herança da Resistência à Mancha bacteriana em Tomateiro. *Fitopatologia brasileira*, v. 30, n. 4, p. 343-349.

Lopes, C. A., Quezado-Duval, M. A. (2005) Doenças bacterianas. In: Lopes, C. A., Ávila, A. C. (ed) *Doenças do tomateiro*. Brasília: Embrapa-CNPH/Embrapa Hortaliças, p. 62-64.

Lopes, C. A., Ávila, A. C. (2002) Informações inadequadas sobre a resistência a doenças em catálogos de cultivares de hortaliças: um exemplo para tomate e pimentão. *Horticultura Brasileira*, Brasília, v. 20, n. 2, p. 130-132.

Lopes, C. A., Quezado-Soares, A. M. (2000) Doenças causadas por bactérias em tomate. In: Zambolim, L., Vale, F. X. R., Costa, H. (ed). *Controle de doenças de plantas: hortaliças.* Viçosa-UFV, p. 754-784.

Malhotra, R. S., Singh, K. B., (1989) Detection of epistasia in chickpea. *Euphytica,* v. 40, p. 169-172.

Marame, F., Fininsa, C., Singh, H., Dessalegne, L., Andersson, A., Sigvald, R. (2010) Occurrence of aphid vector and genetic tolerance to infection by potyvirus in fot pepper. *Euphytica*, v. 171, p. 77-91.

Marcuzzo, L. L., Fernandes, J. M. C, Becker, W. F. (2009) Influência da temperatura e da duração do molhamento foliar na severidade da mancha bacteriana do tomateiro. *Summa Phytopathologica*, v. 35, n. 3, p. 229-230.

Martins, K. C., Pereira, T. N. S., Sousa, S. A. M., Costa, F. R. (2010) Meiose e variabilidade polínica em acessos de *Capsicum annuum* e *Capsicum baccatum. Ciência Rural* [on line], v. 40, p. 746-1751.

Mather, K., Jinks, J. L. (1971) *Biometrical Genetics*. Ithaca, Cornell University Press, p. 382.

Mather, K., Jinks, J. L. (1982) Biometrical Genetics: the Study of Continuous Variation. London UK: *Chapman Hall*. 3 ed.

Menezes, C. B., Maluf, W. R., Faria, M. V., Azevedo, S. M., Resende, J. T. V., Figueira, A. R., Gomes, L. A. A. (2015) Inheritance of resistance to papaya ringspot vírus-watermelon strain (PRSV-W) in Whitaker summer squash line. *Crop Breeding and Applied Biotechnology*, v. 15, p. 203-209.

Minsavage, G. V., Dahlbeck, D., Whalen, M. C., Kearny, B., Bonas, U., Staskawicz, B. J., Stall, R. E. (1990) Gene-for-gene relationships specifying disease resistance in *Xanthomonas campestris* pv. *vesicatoria*–pepper interactions. *Molecular Plant-Microbe Interact*, v. 3, p. 41-47.

Monteiro, E. R., Bastos, E. M., Lopes, A. C. A., Gomes, R. F. L.,Nunes, J. A. R. (2010) Diversidade genética entre acessos de espécies cultivadas de pimentas. *Ciência Rural*, v. 40, n. 2, p. 288-293.

Moraes, I. C. F., Sobral, P. J. A., Branco, I. G. Bazo Ré, T., Gomide, C. A. (2013) Dehydration of “dedo de moça” pepper: kinetics and phytochemical concentration. *Food Science and Technology*, Campinas, fevereiro, v. 33, p. 134–141.

Moreira, G. R., Caliman, F. R. B., Silva, D. J. H., Ribeiro, C. S. C. (2006) *Espécies e variedades de pimenta*. Informe Agropecuário, EPAMIG, Belo Horizonte, v. 27, p. 16-29.

Moreira, S. O., Rodrigues, R., Araújo, M. L., Riva-Souza, E. M., Oliveira, R. L. (2010) Desempenho agronômico de linhas endogâmicas ecombinadas de *Capsicum annuum* L. em sistema orgânico sob cultivo protegido. *Ciância agrotecnica,* v. 34, n. 4, p. 886-891.

Moreira, S. O., Gonçalves, L. S. A., Rodrigues, R., Sudré, C. P., Amaral Júnior, A. T., Medeiros, A. M. (2013a) Correlações e análise de trilha sob multicolinearidade em linhas recombinadas de pimenta (*Capsicum annuum* L.) *Revista Brasileira de Ciência Agrária*, v. 8, n. 1, p. 15-20.

Moreira SO, Rodrigues R, Oliveira HS, Medeiros AM, Sudré CP, Gonçalves LSA (2013b). Phenotypic and genotypic variation among *Capsicum annuum* recombinant inbred lines resistant to bacterial spot. *Genet. Mol. Res.* 12 (2): 1232-1242.

Moreira, S. O., Rodrigues, R., Sudré, C. P., Riva-Souza, E. (2015) Resistência à mancha bacterianaa e características agronômicas de linhas recombinadas de *Capsicum annuum* L. *Revista Brasileira de Ciência Agraria*, v. 10, n. 2, p. 198-204.

Moscone, E. A., Scaldaferro, M. A., Gabriele, M., Cecchinni, N. M., Ehrendorfer, F. (2007) The evolution of chili peppers (*Capsicum* – Solanaceae): a cytogenetic perspective. *Acta Horticulturae*, v.745, p.137-169.

Mulamba, N. N., Mock, J. J. (1978) Improvement of yield potential of the Eto 47 Blanco maize (*Zea Mays* L.) population by breeding for plant traits. *Egytian Journal of Genetics and Cytology*, v. 7, p. 40-51.

Naresh, P., Reddy Krishna, M. K., Reddy Chandra, P. H., Reddy Madhavi, K. (2016) Screening chilli (*Capsicum* spp.) germplasm against *Cucumber mosaic virus* and *Chilli veinal mottle virus* and inheritance of resistance. *European Journal of Plant Pathology*, v. 146, n.3, p. 451–464.

Nascimento, W. M., Dias, D. C. F., Freitas, F. A. (2006) Produção de sementes de pimentas. *Informe Agropecuário*, Belo Horizonte, v. 27, p. 30-39.

Nascimento, N. F. F., Rêgo, E. R., Nascimento, M. F., Finger, F. L., Bruckner, C. H., Silva Neto, J. J., Rêgo, M. M. (2012) Heritability and variability of morphological traits in a segregation generation of ornamental pepper. *Acta Horticulturae*, v. 953, p. 299-304.

Nesi, C. N., *D*al Bó, H. C., Guidoni, A. L., Bringhenti, C. (2010) Número mínimo de repetições em experimentos de competição de híbridos de milho. *Revista de Ciências Agroveterinárias*., v. 9, n. 1, p. 74-81.

Oliveira, F. A., Duarte, S. N., Medeiros, J. F., Oliveira, M. K. T., Silva, R. C. P., Souza, M. S. (2016) Eficiência da fertirrigação nitrogenada e potássica na produção de pimentão cultivado em ambiente protegido. *Revista de Ciências Agrárias*, v. 59 n. 3, p. 263-269.

Oliveira, H. S., Rodrigues, R., Bento, C. dos S., Medeiros, A. M., Sudré, C. P., Couto, M. F., Viana, A. P. (2015) Towards a new strategy to breed an autogamous plant: A case of study in *Capsicum baccatum* var. *pendulum*. *Scientia Horticulturae*, v. 92, p. 279-286.

Oliveira, F. A., Medeiros, J. F., Linhares, P. S. F., Alves, R. C., Medeiros, A. M. M., Oliveira, M. K. T. (2014) Pepper seedlings production fertigated with various nutrient solutions. *Horticultura Brasileira*, v. 32, n. 4, p. 458–463.

Panorama Rural. (2006) Pimenta - um mundo de cores e sabores. *Panorama Rural*, v. 84, p. 30-35.

Paula, F. L. M., Paula, A. L., Frizzone, J. A., Vilas Boas, M. A., Gomes, A. W. A., Souza, C. (2011) Produção de pimenta tabasco com aplicação de CO_2, utilizando-se irrigação por gotejamento. *Acta Scientiarum Agronomy*, março, v. 33, n. 1, p. 133–138.

Paulus, D., Valmorbida, R., Santin, A., Toffoli, E., Paulus, E. (2015) Growth, yield and fruit quality of pepper (*Capsicum annuum*) at different spacings. *Horticultura Brasileira*, março, v. 33, n. 1, p. 91–100.

Pesek, J., Baker, R. J. (1969) Desired improvement in relation to selected indices. Canadian Journal Plant Science, v. 49, p. 803-804.

Pickersgill, B. (1997) Genetic resources and breeding of *Capsicum* spp. *Euphytica*, v.96, n.1, p. 129-133.

Pimenta, S., Rodrigues, R., Sudré, C. P., Moraes, J. G. T., Bento, C. S., Medeiros, A. M. (2016) Protecting vegetable cultivars in Brazil: a chili pepper case-study research. *Horticultura brasileira,* v. 34, p. 161-167.

Pimenta, S. (2015). *UENF Carioca e UENF Carioquinha: novas cultivares de pimenta (Capsicum annuum var. annuum) resistentes à mancha bacteriana*. Tese (Doutorado em Genética e Melhoramento) - Campos dos Goytacazes – UENF, 92p.

Pimentel-Gomes, F. (2009) *Curso de estatística experimental*. Piracicaba: FEALQ, p. 451.

Potnis, N., Krasileva, K., Chow, V., Almeida, N. F., Patil, P. B., Ryan, R. P., Sharlach, M., Behlau, F., Dow, J. M., Momol, M. T., White, F. F., Preston, J. F., Vinatzer, B. A., Koebnik, R., Setubal, J. C., Norman, D. J., Staskawicz, B. J., Jones, J. B. (2011) Comparative genomics reveals diversity among xanthomonads infecting tomato and pepper. *BMC Genomics*, v. 12, p. 146.

Potnis, N., Timilsina, S., Strayer, A., Shantharaj, D., Barak, J. D., Paret, M. L., Vallad, G. E., Jones, J. B. (2015) Bacterial spot of tomato and pepper: diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Molecular Plant Pathology,* December, v. 16, n. 9, p. 907-20.

Quezado-Duval, A. M., Camargo, L.E.A. (2004) Raças de *Xanthomonas* spp. Associadas à mancha bacteriana em tomate para processamento industrial no Brasil. *Horticultura Brasileira*, v. 22, n. 1, p. 80-86.

Quezado-Duval, A. M., Lopes, C. A. (2010) *Mancha-bacteriana: uma atualização para o sistema de produção integrada de tomate indústria*. Circular Técnica (Embrapa Hortaliças), v. 84, p. 1-24.

Rêgo, E. R., Santos, R. M. C., Rêgo, M. M., Nascimento, N. F. F., Nascimento, M. F., Bairral, M. A. (2012) Quantitative and Multicategoric Descriptors for Phenotypic Variability in a Segregating Generation of Ornamental Peppers. *Acta Horticulturae*, v. 93, p. 289-296.

Rêgo, E. R., Finger, F. L., Rêgo, M. M. (2011) *Produção Genética e Melhoramento de Pimentas* (*Capsicum* spp.) Areia-PB, pg. 54.

Rêgo, E. R., Rêgo, M. M., Silva, D. F., Santos, R. M. C., Sapucay, M. J. L. C., Silva, D.R., Silva Júnior, S. J. (2009) Selection for leaf and plant size and longevity of ornamental peppers (*Capsicum* spp.) grown under greenhouse condition. *Acta Horticulturae*, v. 829, p. 371-374.

Reifschneider, F. J. B. (2000) *Capsicum*: *Pimentas e pimentões no Brasil*. Brasília, 113p.

Rezende, J. A. M., Martins, M. C. (2005) Doenças do mamoeiro. In: Kimati, H., Amorim, L., Rezende, J. A. M., Bergamim Filho, A., Camargo, L. E. A. (Ed). *Manual de Fitopatologia*. Doença das plantas cultivadas. São Paulo: v. 2, p. 435-443.

Rezende, V. F. (2004) *Análise de resistência à antracnose foliar em milho*. Tese (doutorado) – Escola Superior de Agricultura Luiz de Queiroz.

Ribeiro, C. S. da C., Cruz, D. M. R. (2004) *Pimentão: tendência de mercado. Cultivar hortaliças e frutas*, Rio Grande do Sul, v. 14, n. 3, p. 16-19.

Ribeiro, E. H., Pereira, M. G., Coelho, K. S., Freitas Júnior, S. P. (2009) Estimativas de parâmetros genéticos e seleção de linhagens endogâmicas recombinantes de feijoeiro comum (*Phaseolus vulgaris* L.). *Revista Ceres*, v. 56, n. 5, p. 580-590.

Ritchie, J. T., Singh, U., Godwin, D. C., Bowen, W. T., Tsuji, G. Y., Hoogenboom, G., Thorton, P. K. (1998) Soil water balance and plant water stress. In: Tsuji, G, Y,; Hoogenboom, G.; Thorton, P. K. *Understanding options for agricultural production*, Kluwer Acad. Pub.

Riva, E. M., Rodrigues, R., Pereira, M. G., Sudré, C. P., Karasawa, M., Amaral Júnior, A. T. do. (2004) Inheritance of bacterial spot disease in *Capsicum annuum* L. *Crop Breeding and Applied Biotechnology*, v.4, n.4,np. 490-494.

Riva-Souza, E. M., Rodrigues R., Sudré, C. P., Pereira, M. G., Viana, A. P., Amaral Júnior, A. T. (2007) Obtaining pepper $F_{2:3}$ lines with resistance to the bacterial spot using the pedigree method. *Horticultura Brasileira,* v. 25, p. 567-571.

Riva-Souza, E. M., Rodrigues, R., Sudré, C. P., Gonzaga, M. P., Bento, C. S., Matta, F. P. (2009) Genetic parameters and selection for resistance to bacterial spot in recombinant F6 lines of *Capsicum annuum*. *Crop Breeding and Applied Biotechnology*, v.9, n.2, p.108-115.

Rodrigues Neto, J., Malavolta Jr., V.A., Victor, O. (1986) Meio simples para o isolamento e cultivo de *Xanthomonas campestris* pv. *citri* tipo B. *Summa Phytopathology*, v. 12, n.6, p. 1-2.

Sahin, F., Miller, S.A. (1998) Resistance in *Capsicum pubescens* to of *Xanthomonas campestris* pv. *vesicatoria* pepper race 6. *Plant Disease*, v.82, n.7, p.794-799.

Santos, M. M. P., Vieira-da-Motta, O., Vieira, I. J. C., Braz-Filho, R., Gonçalves, P. S., Maria, E. J., Terra, W. S., Rosana, R., Souza, C. L. M. (2011) Antibacterial activity of *Capsicum annuum* extract and synthetic capsaicinoid derivatives against Streptococcus mutans. *Natural Medicines*, v. 60, p.1-3.

Schornack, S., Minsavage, G. V., Stall, R. E., Jones, J. B., Lahaye, T. (2008) Characterization of AvrHah1, a novel AvrBs3-like effector from *Xanthomonas gardneri* with virulence and avirulence activity. *New Phytology*, v.179, p. 546-556.

Shaner, G., Finney, R. E. (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. *Phytopathology,* v. 70, p. 1183-1186.

Silva, M. I. S., Bearzoti, E., Bueno Filho, J. S. S. (2009) Análise bayesiana do modelo de herança monogênica no melhoramento vegetal: um exemplo com abobrinha. *Ciência Agrotécnica*, Lavras, v.33, n.6, p. 1463-1468.

Silva, W.P. (2003) *Estimadores de máxima verossimilhança em misturas de densidades normais: uma aplicação em genética*. Lavras. Dissertação (Mestrado) – Universidade Federal de Lavras, p. 60.

Silva-Lobo, V. L., Lopes, C. A., Giordano, L. B. (2005) Herança da resistência à mancha bacteriana e crescimento de *Xanthomonas campestris* pv. *vesicatoria*, raça T2, em genótipos de tomateiro. *Fitopatologia Brasileira*, v. 30, n.1, p. 17-20.

Silva, L. R. A., Rodrigues, R., Pimenta, S., Correa, J. W. S., Araujo, M. S. B., Bento, C. S., Sudré, C. P. (2017) Inheritance of bacterial spot resistance in *Capsicum annuum* var. *annuum. Genetic and Molecular Research,* v. 16, n. 2, p. 1-11.

Silveira, L. M., Queiróz, M. A., Lima, J. A. A., Nunes, G. H. S., Nascimento, A. K. Q., Lima Neto, I. S. (2015). *Revista Ceres,* Viçosa, v. 61, n. 4, p. 530-537.

Smith, H. F. (1936) A discriminant function for plant selection. *Annals of Eugenics,* London, v.7, p. 240-250.

Stall, R. E., Jones, J. B., Minsavage, G. V. (2009) Durability of resistance in tomato and pepper to Xanthomonads causing bacterial spot. *Annual Review of Phytopathology*, v.47, p. 265-84.

Stommel, J.R., Bosland, P.W. (2005) Pepper Ornamental, *Capsicum annuum*. In N.O. Anderson (ed.). Flower breeding and genetics: Issues, challenger and opportunities for the 21st century. *Kluwer Academic Publishers*, The Netherlands.Storck, L., Ribeiro, N. D. (2011) Valores genéticos de linhas puras de soja preditos com o uso do método de Papadakis. *Braganti*, Campinas, v. 4, p. 1-6.

Subandi, W., Compton, A., Empig, L. T. (1973) Comparison of the efficiencies of selection indices for three traits in two variety crosses of corn. *Crop Science*, v. 13, n. 2, p. 184-186.

Sudré, C.P. (2003) *Divergência genética e avaliação da resistência à mancha bacteriana em Capsicum spp.* Dissertação (Mestrado) - Universidade Estadual do Norte Fluminense Darcy Ribeiro, 112 f.

Tavares, E. H., Hemp, S., Canton T. (1999) Análise da interação genótipo x ambiente na avaliação de cultivares de feijão em Santa Catarina. *Pesquisa Agropecuária Gaúcha*, v. 5, n. 271-275.

Teixeira, D. H. L., Oliveira, M. S. P., Goncalves, F. M. A., Nunes, J. A. R. (2012) Índices de seleção no aprimoramento simultâneo dos componentes da produção de frutos em açaizeiro. *Pesquisa agropecuária brasileira*, Brasília, v.47, n.2, p.237-243.

Teodoro, A. F. P., Alves, R. B. N., Ribeiro, L. B., Reis, K., Reifschneider, F. J. B., Fonseca M. E. N., Silva, J. P., Agostini-Costa, T. S. (2013) Vitamin C content in Habanero pepper accessions (*Capsicum chinense*). *Horticultura Brasileira,* v. 31, p. 59-62.

Timilsina, S., Jibrin, M. O., Potnis, N., Minsavage, G. V., Kebede, M., Schwartz, A., Bart, R., Staskawicz, B., Boyer, C., Vallad, G. E., Pruvost, O., Jones, J. B., and Goss, E.M. (2014) Multilocus sequence analysis of xanthomonads causing bacterial spot of tomato and pepper reveals strains generated by recombination among species and recent global spread of *Xanthomonas gardneri. Applied and environmental microbiology*, v. 81, n. 4, p. 1520-1529.

Vallejos, C. E., Jones, V., Stall, R.E., Jones, J. B., Minsavage, G. V., Shultz, D. C., Rodrigues, R., Olsen, L. E., Mazourek, M. (2010) Characterization of two recessive genes controlling resistance to all races of bacterial spot in peppers. *Theoretical and Applied Genetics,* 121: 37-46.

Viñals, F.N., Ortega, R.G., Garcia, J.C. (1996) El cultivo de pimientos, chiles y ajies. Madrid: *Mundi-Prensa*, p. 607.

Vivas, M., Silveira, S.F., Vivas, J.M.S., Pereira, M.G. (2013) Predição de ganhos genéticos e seleção de progênies de mamoeiro para resistência à pinta-preta Marcelo. *Tropical Plant Pathology*, v. 38, n. 2.

Wahyuni, Y., Ballester, A. R., Sudarmonowati, E., Bino, R. J., Bovy, A. G. (2011) Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: Variation in health-related compounds and implications for breeding. *Phytochemistry*, v. 72, n. 11–12, p. 1358–1370.

Wai, K. P. P., Siddique, M. I., Mo, H-S., Yoo, H.J., Byeon, S-E., Jegal, Y., Mekuriaw, A. A., Kim, B-S. (2015) Pathotypes of Bacterial Spot Pathogen Infecting *Capsicum* Peppers in Korea. *Plant Pathology Journal*, v. 31, n.4, p. 428-432

Wesołowska, A., Jadczak, D., Grzeszczuk, M., 2011. Chemical composition of the pepper fruit extracts of hot cultivars *Capsicum annuum* L. *Acta Scientiarum Polonorum: Hortorum Cultus*, v. 10, n. 1, p. 171–184.

Williams, J.S. (1962) The evolution of a selection índex. *Biometrics*, v. 18, p. 375-393.

Zewdie, Y., Bosland, P. W. (2000) Evaluation of genotype, environment, and genotype-by-environment interaction for capsaicinoids in *Capsicum annuum* L. *Euphytica*, v. 111, n. 3, p. 185-190.

Zhang, L., Cheng, X., Gao, Y., Zheng, J., Xu, Q., Sun, Y., Guan,H., Yu, H., Sun, Z. (2015) Apigenin induces autophagic cell death in human papillary thyroid carcinoma BCPAP cells. *The Royal Society of Chemistry*, v. 6, n. 11, p. 3464-72.

Zijlstra, S., Purimahua, C., Lindhout, P. (1991) Pollen tube growth in interespecific crosses between *Capsicum* species. *Hort Science*, v. 26, p. 585-586.

ATTACHMENT

Table A -Table with the gross values of all the characteristics for selection of superior genotypes

Indivíduo	AACPD	CF	DF	EP	TSS	NL	FF	CF	CAPS	AT	VITc	PROD
1	28	53.39	36.11	5.17	6.88	3	5	8	1	8.29	12.69	0.40
2	29	80.47	26.86	2.56	11.28	2	1	8	2	4.00	6.77	0.06
3	28	55.25	29.98	2.19	12.1	2	3	7	1	5.57	25.08	0.61
4	17.5	63.00	28.61	2.49	9.6	2	3	9	1	5.29	73.69	0.81
5	29	65.15	21.66	2.79	8.48	2	1	8	1	5.29	16.15	0.49
6	29	42.77	30.57	3.56	7.62	2	5	8	1	6.14	14.92	0.08
7	22.5	44.89	29.06	2.81	8.44	2	3	8	1	6.00	14.46	0.28
8	29	53.07	32.07	2.36	10.76	2	5	8	1	4.86	2.46	0.33
9	29	64.98	30.62	3.19	9.5	2	3	8	1	4.00	39.69	0.48
10	16	65.22	20.68	2.59	7.5	2	1	8	1	7.71	2.31	0.47
11	22	82.20	32.53	3.12	10.82	2	1	8	2	6.57	80.31	0.82
12	21	78.17	35.10	3.25	8.18	2	3	8	1	7.86	11.08	0.44
13	30	76.35	39.98	3.34	10.48	2	3	8	2	6.86	36.31	0.65
14	19	58.81	26.17	2.79	10.58	3	3	8	1	2.29	16.15	0.90
15	29	57.00	24.68	2.20	12.16	2	3	8	1	5.00	47.08	0.20
16	17	64.55	20.03	2.26	11.32	2	1	8	1	5.29	44.77	0.17
17	30	59.81	31.496	2.67	9.98	3	3	8	1	8.71	49.85	0.53
18	30	64.63	28.71	2.87	9.54	2	1	8	1	5.57	49.38	1.09
19	30	61.26	27.04	2.50	10.3	2	3	9	1	6.71	48.46	0.56
20	28	69.65	39.85	2.74	7.614	2	5	8	2	5.43	27.08	0.07
21	28	40.71	26.34	3.05	8.98	2	3	7	1	7.71	36.00	0.10
22	28	75.41	31.00	3.10	9.52	2	3	8	1	4.71	12.92	1.08

Table A –Cont.

Indivíduo	AACPD	CF	DF	EP	TSS	NL	FF	CF	CAPS	AT	VITc	PROD
23	30	57.11	26.67	2.19	8.9	2	3	8	1	6.29	21.54	0.66
24	24	55.86	30.72	3.24	9.52	3	3	8	1	4.14	30.77	0.73
25	27	79.85	28.98	2.84	9.18	2	1	8	1	5.29	58.77	0.92
26	29	63.27	21.44	2.78	10.38	2	3	8	1	4.86	18.77	0.44
27	21.5	41.70	27.07	2.48	12.96	2	3	8	1	8.43	68.92	0.21
28	31	67.49	26.48	2.62	7.26	2	3	8	1	9.00	68.92	0.50
29	28	51.41	28.69	3.37	8.22	2	3	8	1	4.86	41.54	0.60
30	26	72.40	21.58	1.96	10.26	2	1	8	1	5.43	15.38	1.11
31	27	71.46	37.48	3.35	11.42	3	3	8	2	4.71	45.54	0.81
32	25	68.01	29.68	3.05	7.84	3	3	8	2	4.57	64.15	0.20
33	27	53.01	21.14	1.84	11.84	3	3	8	1	5.29	33.38	0.19
34	18	85.45	36.71	2.27	11.26	2	3	8	1	5.43	40.46	0.60
35	28	84.17	28.03	2.80	8	2	1	8	1	4.29	17.54	0.88
36	26	78.97	32.11	2.93	8.78	2	1	8	1	2.14	2.77	0.65
37	20.5	60.53	23.55	2.10	13.46	2	1	7	1	3.86	61.38	0.20
38	20	100.09	29.03	3.05	9.02	2	1	8	1	8.00	11.38	0.63
39	13.5	63.05	26.20	2.24	10.02	2	3	8	1	8.86	75.08	0.26
40	12.5	48.87	31.45	3.15	10.7	3	5	8	1	6.43	33.08	0.36
41	11.5	52.65	22.17	2.10	9.26	3	3	7	1	4.57	67.69	0.20
42	20	111.86	30.22	3.60	12.76	2	3	8	1	4.71	56.46	0.37
43	15	50.63	25.40	2.98	10.7	2	3	8	1	4.00	55.23	0.63
44	23	53.56	44.51	2.46	8.72	3	5	7	2	2.14	62.15	0.22
45	13.5	56.51	31.43	2.48	10.2	2	3	8	1	6.00	67.38	0.76

Table A –Cont.

Indivíduo	AACPD	CF	DF	EP	TSS	NL	FF	CF	CAPS	AT	VITc	PROD
46	13.5	98.38	19.59	2.26	11.68	2	1	8	1	6.14	30.46	0.77
47	15.5	78.53	26.87	1.75	12.14	2	1	8	1	6.14	8.77	0.29
48	9.5	52.31	39.27	1.99	11.84	3	5	8	1	6.43	46.31	0.43
49	13	48.99	24.74	1.80	7.74	2	5	8	1	5.71	25.23	0.64
50	14	71.45	32.12	2.81	9.46	2	3	8	1	7.29	17.69	0.64
51	20	52.86	26.64	2.37	9.82	2	3	8	1	5.00	63.08	0.35
52	15.5	62.53	31.11	3.99	8.94	2	1	9	1	2.43	78.46	0.42
53	15.5	64.80	26.78	3.00	9.28	2	3	8	1	6.71	33.69	0.63
54	18	87.90	31.46	3.05	9.54	2	3	8	1	3.86	19.69	0.63
55	17.5	68.90	26.32	2.30	8.98	2	1	8	1	5.00	36.00	0.33
56	28	66.47	22.96	3.15	8.98	2	3	8	1	11.29	5.23	0.45
57	15	64.66	35.62	2.85	10.58	3	3	8	1	7.14	73.85	0.89
58	19	71.41	24.43	2.55	10.64	2	3	8	1	7.14	30.00	0.54
59	30	85.13	27.8	2.17	12.92	2	1	8	1	5.43	19.85	0.74
60	15	58.46	28.81	2.25	8.58	2	3	8	1	3.71	54.15	0.50
61	15.5	80.94	25.19	2.03	12.44	2	1	8	1	4.57	72.00	0.38
62	28	60.67	36.23	3.29	8.92	2	3	8	1	5.43	6.15	0.77
63	14.5	37.74	24.68	2.54	12.72	2	3	8	1	3.86	23.38	0.19
64	20	50.57	32.10	3.73	7.98	2	3	8	1	5.00	2.92	0.75
65	13.5	45.85	25.94	1.60	10.64	2	3	8	1	2.71	20.46	0.17
66	26	52.69	31.50	2.86	11.12	3	3	8	1	5.86	61.54	0.13
67	15	69.83	28.83	2.83	7.5	2	3	8	1	4.57	48.15	0.46
68	15.5	63.28	26.92	2.09	12.18	3	3	8	2	5.57	31.54	0.24
69	28	60.78	24.61	2.63	12.38	3	1	8	2	5.86	73.54	0.30

Table A –Cont.

Indivíduo	AACPD	CF	DF	EP	TSS	NL	FF	CF	CAPS	AT	VITc	PROD
70	17	68.07	23.60	1.92	12.94	2	1	8	2	3.71	66.15	0.39
71	24	62.73	29.70	2.61	11.66	2	3	8	1	4.14	53.69	0.47
72	15	73.80	25.09	2.77	11.26	2	1	8	1	2.43	61.54	0.23
73	18	70.99	40.00	4.27	8.26	2	5	8	1	4.71	15.69	0.11
74	17	36.70	32.11	2.99	11.26	2	3	8	1	4.00	36.62	1.04
75	18	73.24	29.90	2.96	8.84	2	3	8	1	4.43	57.54	0.22
76	17	75.64	38.67	4.44	7.72	3	5	8	1	3.57	67.23	0.79
77	18.5	73.40	27.20	2.34	10.14	2	1	8	1	5.43	10.46	0.82
78	26	73.54	24.36	2.51	6.42	2	3	7	1	4.14	78.92	0.34
79	23	73.34	27.41	3.04	8.86	3	1	8	2	4.86	68.77	0.48
80	21	61.88	28.29	3.03	9.16	2	3	8	1	7.00	12.62	0.58
81	18	74.16	23.14	2.28	11.4	2	1	8	1	3.71	68.77	0.53
82	27	33.16	26.76	2.47	8.64	3	3	8	1	6.14	55.08	0.12
83	27	58.13	26.26	2.20	11.44	2	3	8	1	4.29	47.69	0.55
84	21	79.28	30.14	2.84	8.94	2	3	8	1	7.43	16.62	0.54
85	17	106.67	32.71	2.36	8.8	2	1	8	2	3.14	77.08	0.55
86	23	52.35	25.45	2.11	11.32	2	3	8	1	4.29	60.62	0.21
87	13.5	91.58	24.81	2.37	11.4	2	1	8	1	4.57	40.77	0.59
88	19	70.40	31.63	3.19	10.44	2	3	8	1	5.43	11.23	0.29
89	21	73.48	27.76	2.20	10.58	2	3	8	2	5.14	41.23	1.05
90	20	63.31	34.09	2.82	9.76	2	5	8	1	6.43	49.54	0.45
91	25	55.12	35.96	2.40	12.1	2	3	8	2	5.86	64.92	0.58
92	27	70.45	26.73	2.64	9.7	2	3	8	2	5.29	58.92	0.53
93	13.5	63.21	23.45	2.11	9.64	2	3	8	2	4.29	20.77	0.83

Table A –Cont.

Indivíduo	AACPD	CF	DF	EP	TSS	NL	FF	CF	CAPS	AT	VITc	PROD
94	15.5	102.71	28.92	3.09	7.48	2	1	8	1	3.71	27.69	0.44
95	26	80.94	30.13	2.42	7.98	2	3	8	2	5.86	53.85	0.94
96	27	69.08	27.29	2.71	10.02	2	3	8	1	4.00	66.15	0.38
97	14.5	92.34	49.80	3.64	8.16	3	5	8	1	6.86	40.00	1.38
98	15.5	79.48	30.28	2.65	8.8	2	3	8	1	4.86	40.00	0.53
99	14.5	83.43	23.30	1.78	10.3	2	1	8	1	4.29	78.92	0.54
100	26	72.85	20.57	2.28	6.8	2	3	8	1	5.14	11.69	0.41
101	22	49.61	28.57	2.46	11.72	2	3	8	1	7.00	51.69	0.58
102	16	83.58	30.34	2.49	10.06	3	3	8	1	8.00	72.31	0.46
103	16.5	73.09	32.58	3.23	8.82	3	3	9	1	3.57	61.69	0.45
104	17.5	54.44	24.26	2.18	11.68	2	3	8	1	8.14	4.62	0.32
105	17.5	41.78	26.47	2.34	8.16	2	5	8	1	6.29	23.85	0.42
106	17.5	77.22	30.28	2.79	9.18	2	3	8	1	6.00	7.69	0.99
107	26	63.91	33.84	2.80	8.3	2	3	8	2	6.57	32.15	0.49
108	26	83.85	42.59	2.58	9.22	3	3	8	1	6.57	36.46	0.73
109	15.5	66.78	20.92	2.01	8.82	2	1	9	1	6.71	63.54	0.48
110	15.5	58.23	21.31	1.78	8	2	3	8	1	10.86	30.77	0.34
111	22	57.37	24.05	2.31	9.68	2	3	8	1	5.43	5.23	0.58
112	19	54.30	16.61	1.93	9.6	2	1	7	1	4.43	52.62	0.30
113	29	55.85	20.28	2.35	10.86	2	1	8	1	3.57	40.62	0.34
114	22	93.13	29.48	2.48	7.84	2	1	8	1	6.43	47.38	0.45
115	19	85.13	28.77	2.49	9.06	2	1	8	1	5.71	54.92	0.44
116	16	83.43	26.71	2.86	11.16	2	1	8	1	3.57	54.62	0.45
117	17	53.75	31.08	3.24	10.52	2	3	8	2	4.43	22.62	0.52

Table A –Cont.

Indivíduo	AACPD	CF	DF	EP	TSS	NL	FF	CF	CAPS	AT	VITc	PROD
118	18	56.91	30.71	2.51	10.52	3	3	8	1	5.14	65.54	0.69
119	28	52.57	24.94	2.07	12.76	2	3	8	1	5.00	40.77	0.27
120	15.5	56.33	29.98	2.33	7.925	3	3	8	1	5.71	34.92	0.03
121	15.5	50.63	21.04	2.04	12.76	3	1	8	1	6.00	83.08	0.86
122	18	55.44	30.8	2.74	9.02	3	5	8	1	5.86	64.77	2.84
123	15.5	88.08	28.22	2.49	13	2	1	8	1	7.00	74.46	0.20
124	10.5	65.24	28.88	2.70	10.66	2	3	8	1	5.43	4.46	0.35
125	15.5	68.44	30.30	3.51	7.96	2	3	9	2	3.57	60.00	0.14
126	28	68.21	27.95	2.52	9.04	2	3	8	2	5.29	23.38	0.55
127	28	55.00	34.74	2.99	9.98	2	3	7	1	6.43	68.00	0.38
128	19	66.98	30.18	3.40	8.34	2	1	8	1	4.43	78.46	0.41
129	27	61.35	27.61	2.70	9.02	2	3	9	1	3.57	64.15	0.34
130	28	92.45	27.32	2.32	10.56	2	1	8	2	4.14	51.85	0.21
131	26	88.84	22.83	2.48	12.64	2	1	8	2	6.14	5.38	0.25
132	28	50.80	31.04	3.19	7.98	2	3	8	1	6.14	36.62	0.34
133	20	42.17	28.19	1.98	9.48	3	3	8	1	3.71	32.77	0.24
134	16	62.77	31.90	3.414	10.4	3	3	8	1	6.14	60.15	0.44
135	26	64.50	25.06	2.36	7.08	2	3	8	1	4.29	17.38	0.39
136	28	83.10	28.27	2.39	10.14	3	1	8	2	3.86	16.77	1.03
137	14.5	77.67	36.78	3.27	8.96	3	5	8	1	6.14	71.85	0.42
138	22	47.68	24.49	3.27	8.56	2	5	9	1	1.29	51.69	0.79
139	25	60.13	19.29	2.17	10.4	2	1	8	1	9.00	75.69	0.12
140	20.5	41.48	25.48	1.65	8.48	3	5	8	1	4.14	37.69	0.46
141	26	86.13	29.10	2.60	10.9	3	1	8	2	6.43	36.92	0.64

Table A –Cont.

Indivíduo	AACPD	CF	DF	EP	TSS	NL	FF	CF	CAPS	AT	VITc	PROD
142	20	74.57	29.32	2.55	11.44	2	3	8	2	5.29	16.15	0.35
143	19	55.39	37.96	3.39	9.74	3	5	8	1	8.57	58.00	0.22
144	21	50.00	30.81	3.01	9.06	2	5	8	1	2.43	67.69	0.33
145	20	47.79	20.39	3.10	9.825	2	3	8	1	5.43	48.31	0.34
146	14.5	65.30	25.07	2.12	10.68	2	1	8	1	5.43	47.54	0.27
147	25	47.54	29.23	2.92	8.94	3	5	8	1	7.71	44.92	0.76
148	23	32.34	31.93	2.24	8.84	3	5	8	1	7.00	31.54	0.08
149	28	69.47	26.40	3.33	10.68	3	1	8	1	4.14	53.85	0.35
150	15.5	64.06	35.40	3.76	9.24	2	3	8	1	8.29	40.62	0.50
151	15.5	48.68	20.50	1.80	11.84	2	1	8	1	10.71	58.31	0.21
152	13.5	83.15	27.88	2.98	7.32	2	3	8	2	3.29	23.85	0.17
153	25	67.81	28.00	2.26	10.2	2	5	8	2	4.43	22.15	0.54
154	15.5	52.29	22.07	1.90	11.52	2	3	8	1	5.29	47.23	0.30
155	15.5	56.46	27.63	2.70	10.12	2	3	8	1	4.00	54.15	0.53
156	15.5	36.65	20.85	2.00	11.26	2	3	8	1	4.29	13.69	0.23
157	16	60.25	31.84	3.32	8.54	2	3	8	1	6.86	11.54	0.48
158	19	79.33	29.23	3.98	10.34	3	3	8	1	2.71	29.38	0.18
159	26	58.95	29.96	2.27	10.36	2	3	8	2	1.29	10.62	0.33
160	22	71.79	36.13	3.82	8.02	2	3	8	2	4.86	28.92	0.34
161	17.5	74.12	25.45	2.46	9.16	3	1	8	2	4.43	63.69	0.52
162	26	53.42	32.25	3.55	8.88	3	5	8	2	5.71	41.85	0.56
163	27	44.93	32.88	3.86	9.16	2	5	7	1	4.57	46.92	0.2
164	22	39.38	31.64	2.98	7.56	3	5	8	1	3.29	51.08	0.34
165	27	39.95	29.26	2.27	10.52	2	5	8	2	6.43	33.69	0.17

Table A –Cont.

Indivíduo	AACPD	CF	DF	EP	TSS	NL	FF	CF	CAPS	AT	VITc	PROD
166	13.5	74.84	27.57	3.22	10.52	2	3	8	1	4.57	52.00	0.24
167	28	56.89	28.19	3.62	7.9	2	3	8	1	7.00	15.85	0.42
168	27	65.23	24.63	2.69	9.06	2	1	8	1	9.43	26.15	0.45
169	28	37.18	19.81	2.37	8.8	2	3	8	2	6.43	30.62	0.14
170	16	71.19	21.28	2.42	9.12	2	1	7	1	4.71	61.08	0.34
171	23	67.35	25.90	3.002	9.18	2	3	8	2	2.71	73.38	0.46
172	15.5	49.28	32.62	11.95	9.28	2	3	8	1	6.14	38.62	0.38
173	20	53.30	24.12	2.26	8.76	3	3	7	2	4.57	23.85	0.22
174	15.5	46.97	22.03	2.18	8.98	2	3	8	1	7.43	37.38	0.17
175	33	37.85	21.58	2.22	11.4	3	3	7	1	6.57	42.92	0.16
176	28	54.01	29.06	3.13	9.32	2	3	8	1	5.43	69.54	0.23
177	17	69.38	21.74	2.01	13.92	2	1	8	1	4.14	41.38	0.17
178	29	45.30	20.17	2.14	12.88	2	3	7	1	9.00	61.69	0.31
179	13.5	33.60	23.50	1.28	14.06	3	3	8	1	6.71	21.08	0.11
180	28	40.97	38.80	3.52	8.74	3	3	8	1	8.29	73.85	0.14
181	13.5	44.14	13.54	1.67	13.3	2	1	8	1	4.14	74.46	0.06
182	22	49.19	26.50	1.748	9.92	2	3	8	1	4.14	4.92	0.23
183	18	31.05	19.22	2.566	11.38	2	3	7	1	6.14	27.08	0.18
184	20	54.76	29.73	2.239	10.79	3	3	7	2	6.14	50.00	0.21
185	17	63.99	30.45	2.55	10.78	2	3	8	1	5.57	19.23	0.26
186	18	62.71	33.37	3.31	6.86	2	3	8	1	6.14	24.00	0.43
187	20	51.72	27.07	3.65	8.86	2	3	8	2	6.86	35.38	0.62
188	15.5	51.72	27.07	3.65	8.86	2	3	8	1	5.14	62.31	0.63
Máximo	33	111.8	49.80	11.95	14.06	-	-	-	-	11.28	83.07	2.84
Mínimo	9.5	31.05	13.54	1.28	6.42	-	-	-	-	1.28	2.30	0.03
Média	21.25	71.45	31.67	6.615	10.24	-	-	-	-	6.28	42.69	1.43