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Resistance to anthracnose (*Colletotrichum scovillei*) in *Capsicum annuum*: inheritance, QTL identification and progenies selection to develop resistant cultivars

Ingrid Gaspar da Costa Geronimo[1∞], Paola Alvares Bianchi[2∞], Maria do Socorro Bezerra de Araújo[3], Antonio André da Silva Alencar[1], Lígia Renata Almeida da Silva[4], Cláudia Pombo Sudré[1], Rosimara Barboza Bispo[1], Helaine Christine Cancela Ramos[1], Rosana Rodrigues[1,*]

[1] *Laboratório de Melhoramento Genético Vegetal, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, RJ, Brazil.*

[2] *GDM Seeds, Palmas, TO, Brazil.*

[3] *Laboratório de Melhoramento Genético Vegetal, Universidade do Estado de Mato Grosso, Cáceres, MT, Brazil.*

[4] *Bayer, Porto Nacional, TO, Brazil.*

[∞] *First and second author contributed equally for this work.*

* *Corresponding author: rosana@uenf.br*

Abstract: Developing sweet and chili peppers resistant cultivars to anthracnose is a challenge for plant breeders inciting the search for resistance sources, inheritance studies, identification of loci, and recombinant inbred resistant lines to fight the disease. In this report, we determined the resistance to anthracnose inheritance in *Capsicum annuum* L. and identified QTL in unripe fruits. The F_1, F_2, BC_1 and BC_2 generations were obtained from the crossing between genotypes UENF 2285 and UENF 1381. The resistance variables evaluated were area under the disease progress curve, incubation period, and latent period in unripe fruits. Disease assessment was performed using a grading scale for a period of seven days after inoculation. For the QTL identification, resistance evaluation was associated with previously mapped molecular markers. At least six genes control the resistance, with one gene with greater effect are responsible for anthracnose resistance. Partial dominance and the additive-dominant model explained the genetic control of the resistance. Six minor QTLs were identified for resistance to anthracnose in the unripe fruit, explaining 23.16% of trait variation. The joint selection of anthracnose resistance and fruit variables resulted in the selection of 60 promising progenies to continue the breeding for sweet pepper resistance to *Colletotrichum scovillei*.

Keywords: Pepper breeding, disease resistance, mean generation analysis, selection index, QTLs mapping

Introduction

Anthracnose is considered one of the main diseases in plants. In 2012, the genus *Colletotrichum* spp. was ranked eighth in the 'Top 10' of pathogenic fungi based on economic and scientific importance (Dean et al., 2012). In *Capsicum* spp. anthracnose can be caused by 35

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Colletotrichum species (Sharma et al., 2022; Araújo et al., 2023; Nisa et al., 2023; Zhang et al., 2023), with the prevalence of species from *acutatum*, *truncatum* and *gloeosporioides* complexes. Among these species, 38% of the literature reports that the most economic damage in the world is caused by *C. scovillei*, *C. truncatum* and *C. siamense* (Araújo et al., 2023). In general terms, anthracnose is characterized by necrotic and depressive lesions in the tissue, with defined borders, concentric rings of acervuli, and conidial mass ranging from withish to orange, usually salmon and rarely brown (Damm et al., 2012; Silva et al., 2014; Almeida et al., 2017), infecting leaves, fruits, stems and roots. It can also cause stem and crown rot and seed blight (Damm et al., 2012).

In *Capsicum* spp. anthracnose mainly affects fruits, at all phenological stages, in the pre- and post-harvest periods (Mahasuk et al., 2009a). Infection and disease development can occur in the field, during transport and the period of sale on previously contaminated shelves, with the possibility of even cross-infection. Often, the visible symptoms of the disease only appear in the final consumer's environment.

Although there are several recommendations for anthracnose control in *Capsicum* spp., such as biological control (Boukaew et al., 2024), systemic abiotic resistance inducers (Jayapala et al., 2020), fungicides (Padghan et al., 2023; Nawaz et al., 2024), cultural management (Islam et al., 2020), genetic resistance (Almeida et al., 2020) is a pillar of integrated disease management. This reinforces the importance of obtaining sources of resistance and understanding the inheritance of this characteristic to define breeding methods to be used in the development of anthracnose-resistant pepper cultivars.

The use of resistant plants has been shown to be an efficient, cost-effective, competitive and non-polluting method of disease control (Monroy-Barbosa and Bosland, 2011). However, for the *Capsicum-Colletotrichum* pathosystem there is still no sweet pepper cultivar with resistance to *C. scovillei* available, although sources of resistance to anthracnose have already been identified as accession PBC932 (*C. chinense*) resistant to *C. capsici* (Mahasuh et al., 2009a), *C. acutatum* (Sun et al., 2015), and *C. truncatum* (Mahasuk et al., 2016); accessions UENF 1718 and UENF 1797 (*C. baccatum* var. *pendulum*), resistant to *C. gloeosporioides* (Silva et al., 2014), and accession UENF 1381 resistant to *C. gloeosporioides* (Bento et al., 2017) (reclassified as *C. scovillei* (Almeida et al., 2020; Giacomin et al., 2020). In November 2019, Feltrin made available a new Biquinho pepper cultivar, *C. chinense*, with resistance to anthracnose caused by *Colletotrichum gloeosporioides*, called Maria Bonita (Feltrin, 2024).

Genetic control for resistance to *Colletotrichum* spp. is highly variable, depending on a number of factors, such as the species of the pathogen, the source of resistance, the methods of inoculation and evaluation, and the stage of fruit development (Araújo et al., 2023).

A plant breeding program aimed at disease resistance is based on genetic inheritance. For anthracnose, several studies have already been conducted in different genotypes/species of *Capsicum* (Figure 1). Seventeen genes have been described and linked to anthracnose resistance (*Arn1*, *Arn2*, *Arn3*, *Arn4*, *Arn5*, *co1*, *co2*, *co3*, *co4*, *Co5*, *RCt1*, *LYM2*, *CQW23_09597*, *CLF*, *NFXL1*, *PR-14* and a candidate gene *CA05g17730*) (Park et al., 1990; Lin et al., 2002; Pakdeevaraporn et al., 2005; Wang and Bosland, 2006; Mahasuk et al., 2009a; Mahasuk et al., 2009b; Mishra et al., 2019a; Zhao et al., 2020; Kethom et al., 2023) with prevalence of dominant genes.

Quantitative inheritance has also been identified in the *Capsicum-Colletotrichum* pathosystem (Mahasuk et al., 2016; Zhao et al., 2020). The identification and location of specific loci mediating quantitative characters is an approach of great importance in plant breeding that aims to expand the knowledge of the genetic inheritance of the characters and identify molecular markers. These markers can be used in assisted selection for relevant phenotypic characteristics, in addition to leading to a better understanding of the interaction between genotype and phenotype.

When the markers linked to the characteristic of interest are identified using computational tools, it is possible to select individuals based on the genotype, a technique known as Marker-Assisted Selection (Lannou, 2012; Na Jinda et al., 2023).

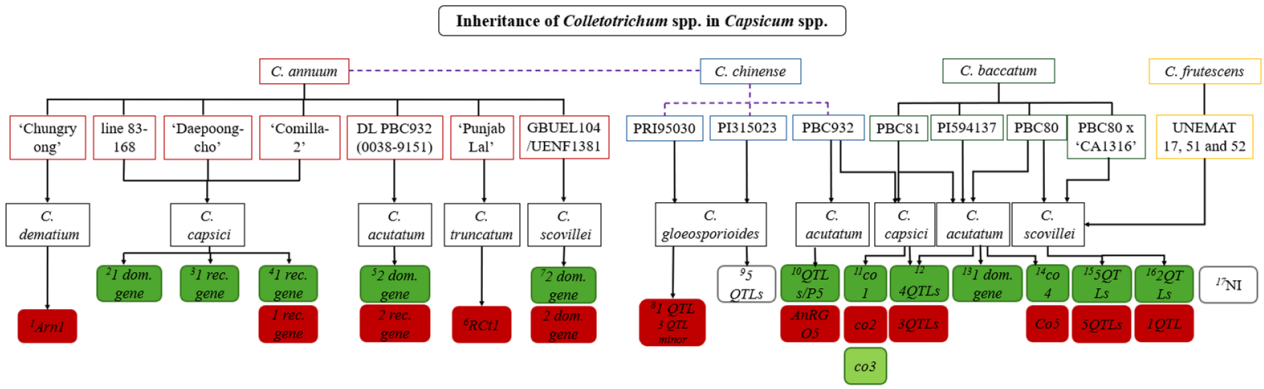

Figure 1. Scheme on the inheritance of resistance to anthracnose (*Colletotrichum* spp.) in *Capsicum* spp. with respective sources of resistance and related genes or QTLs. Genes and QTLs identified in immature and mature fruits and seedlings correspond to the text boxes in dark green, red and light green. The genes or QTLs of articles that did not mention the development stage of the fruit or seedling were left in blank text boxes. Adapted from Araújo et al. (2023).

[1]Park et al., 1990b; [2]Kanchana-Udomkan et al., 2004; [3]Kim et al., 2008a; [4]Rahman and Akanda, 2022; [5]Lin et al., 2007; [6]Mishra et al., 2019b; [7]Giacomin et al., 2020; [8]Voorips et al., 2004; [9]Sanjaya et al., 2002; [10]Mahasuk et al.,2009a, [11]Mahasuk et al., 2016; [12]Lee et al., 2010, [13]Lee et al., 2011; [14]Kim et al., 2008b; [15]Mahasuk et al., 2009b; [16]Suwor et al., 2017; [17]Amorim et al., 2021.

In *Capsicum*, studies have been carried out involving the identification of QTLs associating genomic regions with disease resistance, such as the *C. chinense* resistant to *C. gloeosporioides* (Sunjaya et al., 2002; Voorrips et al., 2004), to *C. capsici* (Voorips et al., 2004), to *C. acutatum*, more specifically on P5 chromosome (Sun et al., 2015; Zhao et al., 2020), and to *C. truncatum* (Mahasuk et al., 2016). In *C. baccatum*, quantitative resistance to *C. acutatum* was reported by (Yoon et al., 2009) and (Lee et al., 2010, 2011); to *C. scovillei* in QTLs on chromosome P2 (Mahasuk et al., 2016) and *C. capsici* in resistance-related QTLs by (Lee et al., 2010, 2011). For the *C. annuum* - *C. scovillei* pathosystem, two independent genes were determined with a polygenic effect for both immature and mature fruits (Giacomin et al., 2020).

Six genotypes of *C. annuum* resistant to anthracnose have been used in inheritance studies (Park et al., 1990b; Lin et al., 2002; Kanchana-udomkan et al., 2004; Kim et al. 2008a; Mishra et al., 2019a; Giacomin et al., 2020; Rahman and Akanda, 2022). Lines of *C. annuum* derived from other resistant species such as PBC 80 (*C. baccatum*) and PBC 932 (*C. chinense*) were also crossed to study inheritance (Kim et al., 2007; Lin et al., 2007; Suwor et al., 2017). However, an analysis of the experimental conditions in these different works revealed a variety of evaluation methods and criteria. Weak criteria, such as symptom assessment for a period of less than seven days, can result in the selection of genotypes with unstable resistance, which under pressure can cause resistance to break down. The breakdown of resistance to anthracnose in some genotypes such as line 83-168 considered resistant in inheritance studies (Lin et al., 2002; Kanchana-udomkan et al., 2004), already had been reported. Differential reactions to *Colletotrichum* spp. was described in the line 83-168, considered as highly susceptible to isolates of *C. capsici*, *C. gloeosporioides* and *C. acutatum* species in the immature and mature stages (Mongkolporn et al., 2010).

Among the genotypes of *C. annuum*, UENF 1381 stands out, resistant to *Xanthomonas euvesicatoria*, to Pepper yellow mosaic virus (PepYMV) and resistant, in immature fruits and moderately resistant to mature fruits, to isolate #8.1 (*C. scovillei*) (Bento et al., 2017; Almeida et al., 2020; Giancomin et al., 2020).

The search for anthracnose-resistant sweet pepper (*C. annuum*) and chili pepper (*Capsicum* spp.) cultivars have challenged breeders and plant pathologists in identifying sources of resistance, in inheritance studies and in breeding programs. In this work, we report the inheritance of resistance to anthracnose (*C. scovillei*) in *C. annuum*, based on phenotyping, genotyping and QTL mapping, considering the reaction to anthracnose at the unripe fruit stage.

Material and Methods

Developing segregant populations

The F_1, F_2, BC_1 and BC_2 generations were obtained from two genotypes of *C. annuum* var. *annuum*, identified in the germplasm bank of the Universidade Estadual do Norte Fluminense Darcy Ribeiro as UENF 2285, used as female parent and UENF 1381, used as male parent. The parent UENF 2285 is a pure line of sweet pepper, therefore without pungency, susceptible to anthracnose. Parental UENF 1381 is a jalapeño-type, pungent, anthracnose-resistant chili pepper accession, caused by *C. scovillei* (Bento et al., 2017; Almeida et al., 2010; Giacomin et al., 2020).

To carry out the crosses, the parents were seeded in 128-cell polystyrene trays and when the seedlings reached four permanent leaves, they were transplanted to pots with a capacity of five liters containing a mixture of soil, sand and manure (1:1:1) and kept in a greenhouse. Crossings were performed in the morning (until 10:00 a.m.) or in the late afternoon (after 4:00 p.m.), in flower buds emasculated in pre-anthesis, identified and covered with paper bags in order to avoid cross-pollination. When ripe, the fruits were harvested and the seeds removed manually (Silva et al., 2017). After obtaining the F_1 generation, this was backcrossed with the respective parents to obtain BC_1 and BC_2 and self-fertilized to obtain the segregating F_2 generation.

Reaction to *Colletotrichum scovillei*

Inoculations were performed with isolate #8.1 from the Laboratório de Melhoramento Genético Vegetal - UENF, which is highly pathogenic (Bento et al., 2017). The isolate was initially identified as *C. gloeosporioides* by the URM (University Recife Micology) and when transferred to the Universidade Estadual de Londrina, it was reclassified by sequencing to *C. scovillei*, named in this institution as UEL8.1U (NCBI GenBank accession numbers MN121780, MN121791, MN121802, MN121811 and MN121822) (Giacomin et al., 2020).

The isolate was kept on Potato-Dextrose-Agar medium for seven days in BOD (Biochemical Oxygen Demand) incubator, at 28 ºC with a photoperiod of 16:8 h (light: dark). After incubation, the conidia suspension was prepared using 10 mL autoclaved deionized water and a drop of Tween 20. The density of the inoculum suspension was adjusted to $1x10^6$ conidia.mL^{-1} with a Neubauer chamber and optical microscope.

To analyze the reaction to the isolate of *C. scovillei*, three immature fruits of each plant of each population were used, totaling 834 fruits, being 39, 48, 42, 93, 90 and 522 fruits of P_1, P_2, F_1, BC_1, BC_2 and F_2, respectively. As a negative control, 288 fruits, one from each plant, were inoculated with autoclaved deionized water. The pedicels and calyx were removed, in order to minimize contamination, and the disinfestation was carried out with immersion in 70% alcohol for one minute, followed by immersion in 0.2% sodium hypochlorite solution for five minutes and then triple washed in autoclaved deionized water. The fruits were dried and placed in Styrofoam trays adhered to by a double-sided tape, in order to avoid the movement of the fruits and the consequent draining of the conidial suspension.

Inoculation was performed with 10 µL of inoculum suspension (1×10^6 conidia.mL^{-1}) placed on a wound made on the central surface of the fruit with an entomological needle. After inoculation, the fruits were kept in a humid chamber. The assessment was performed daily for seven days after inoculation, using a severity score scale proposed by Montri et al. (2009). The resistance level scores ranged from 0 to 9, corresponded to: 0 = HR, highly resistant (no infection); 1 = R, resistant (1–2% of fruit area shows necrotic lesion or a larger lesion soaked in water

around the site of infection); 3 = MR, moderately resistant (>2–5% of fruit area shows necrotic lesion, acervuli may be present or lesion soaked in water up to 5% of fruit surface up to 25% of fruit surface); 7 = S, susceptible (>15–25% of the fruit area has a necrotic lesion with acervuli); and 9 = HS, highly susceptible (>25% of the fruit area shows necrosis, lesion often surrounding the fruit; abundant acervuli).

Variables analyzed

The incubation period (IP) and latent period (LP) were evaluated. The IP corresponds to the period, in days, between inoculation and the onset of disease symptoms, and the LP to the period, in days, between inoculation and the appearance of signs, that is, of the pathogen's reproductive structures, in this case acervuli. From the scores obtained during the seven days of evaluation (Montri et al., 2009), the area under the disease progress curve (AUDPC) was calculated according to Campbell and Madden (1990).

Quantitative analysis

Plants were considered resistant or susceptible, according to the AUDPC values. Considering the scale proposed by Montri et al. (2009), this range was divided into six reaction categories, highly resistant – HR, resistant – R, moderately resistant – MR, moderately susceptible – MS, susceptible – S and highly susceptible – HS. With the values of frequencies observed for these classes, the chi-square test (χ^2) was performed, considering the segregation rate of resistant to susceptible plants in the F_2 and BC_1 generations. The null hypothesis (H_0) was accepted or not, based on the comparison between the calculated χ^2 value (observed frequency) and the tabulated χ^2 value (calculated frequency) at a 5% significance level and the degree of freedom (K-1). Analyzes were performed using the GENES software (Cruz, 2016). The different phenotypic proportions in the F_2 population were analyzed for inheritance conditioned by one gene (3:1) and confirmed for one gene (1:1) in the susceptible backcross population. The null hypothesis implies in obtaining expected segregations equal to those observed, at 5% probability.

Estimates of genetic parameters based on variance components

The AUDPC values were used to estimate the genetic and environmental components and perform the analysis of generation means to estimate the genetic effects involved in the expression of traits (Cruz et al., 2014). Analyzes were performed in the GENES software (Cruz, 2016). The following genetic parameters were estimated:

- Phenotypic variance (σ_p^2): $\sigma_p^2 = \sigma_{F_2}^2$;
- Environmental variance (σ_e^2): $\sigma_e^2 = \frac{(\sigma_{P1}^2 + 2\,\sigma_{F1}^2 + \sigma_{P2}^2)}{4}$;
- Genotypic variance (σ_g^2): $\sigma_g^2 = \sigma_{F2}^2 - \sigma_e^2$;
- Additive variance (σ_a^2): $\sigma_a^2 = 2\sigma_{F_2}^2 - (\sigma_{RC1}^2 + \sigma_{RC2}^2)$;
- Variance due to dominance deviations (σ_d^2): $\sigma_d^2 = \sigma_g^2 - \sigma_a^2$;
- Broad sense heritability (H^2): $H^2 = \frac{\sigma_g^2}{\sigma_{F_2}^2}$;
- Narrow sense heritability (h^2): $h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_d^2 + \sigma_e^2}$;
- Dominance mean degree (DMG): $DMG = \frac{d}{a}$;
- Minimum number of genes (η): $\eta = \frac{R^2\,(1 + 0{,}5k^2)}{8\,\sigma_g^2}$;

Which: σ_{P1}^2 = variance of P_1 generation; σ_{P2}^2 = variance of P_2 generation; σ_{F1}^2 = variance of F_1 generation; σ_{F2}^2 = variance of F_2 generation; σ_{BC1}^2 = variance of BC_1 generation; σ_{BC2}^2 = variance of BC_2 generation; R^2 = full range in F_2 generation; K = DMG.

Estimation of genetic parameters based on generation means

The additive-dominant (*m* + *a* + *d*) and the complete (*m* + *a* + *d* + *yy* + *ad* + *dd*) models were tested. The sum of squares of the parameters was decomposed into sums of squares for each individual effect, and the adequacy of the complete and dominant ad-

ditive model was evaluated using the coefficient of determination (R^2) to assess the contribution of gene effects on the trait under study (Cruz et al., 2014). The following genetic parameters based on generation means were estimated:

$$\underline{P_1} = m + a + aa$$

$$\underline{P_2} = m - a + aa$$

$$\underline{F_1} = m + d + dd$$

$$\underline{F_2} = m + \frac{1}{2}d + \frac{1}{4}dd$$

$$\underline{BC_1} = m + \frac{1}{2}a + \frac{1}{2}d + \frac{1}{4}aa + \frac{1}{4}ad + \frac{1}{4}dd$$

$$\underline{BC_2} = m - \frac{1}{2}a + \frac{1}{2}d + \frac{1}{4}aa - \frac{1}{4}ad + \frac{1}{4}dd$$

Which: m = mean of all possible homozygotes; a = additive effects; d = dominance effects; aa = epistatic additive x additive interaction; ad = epistatic additive x dominance effects; dd = epistatic dominant x dominant interaction.

Selection of F_2 individuals

To proceed with the breeding program targeting *Colletotrichum*-resistant sweet pepper lines, the Mulamba and Mock selection index was used with a selection pressure of 35% (Mulamba and Mock, 1978). This index is based on the sum of ranks, which consists of classifying the genotypes in relation to each of the evaluated characteristics, increasing the mean of the improved population as a result of the distribution of gains between the variables considered. The use of simultaneous selection of characters increases the chance of success in programs that target resistance with agronomic characteristics.

Resistance variables to *C. scovillei* represented by AUDPC, IP, LP were used together with two morphometric variables, fruit diameter (FD) and fruit length (FL), both analyzed in the same study population (Silva, 2018), in the selection index aiming to rank the most resistant genotypes to anthracnose and with larger fruits, that is, with proportions close to the characteristics of sweet pepper.

QTL identification

The anthracnose resistance on unripe fruits of the F_2 generation were used to identify regions with possible QTLs. The Lilliefors test was performed first in order to check the normality of the dataset. The models described by Broman and Sen (2009), in which each genotype of the marker loci was adjusted as an effect of covariates against the measured phenotypic variables, were used to identify the QTLs. Standard interval mapping methods were used, which allowed verification of the position of possible QTLs along the linkage map obtained by Bianchi (2021), using ISSR (Inter Single Sequence Repeat), microsatellites (SSR – Single Sequence Repeat) and AFLP (Amplified Fragment Length Polymorphism) markers in the F_2 generation of *C. annuum* studied in this work.

The interval-mapping methods made it possible to observe the position of possible QTL throughout the linkage map, which was estimated by the following premise:

$$P_{ij} = Pr\left(g_i = {}_j / M_i\right)$$

where: P_{ij} = probability of existence of QTL; g_i = possible genotype of the QTL; j = measured phenotypic variable; and M_i = genotype of the marker locus.

The lod scores were obtained by the following estimator:

$$LOD = Log10\left(\frac{\prod_i \sum_{jpi} \Phi(y_i, \hat{\mu}_i, \hat{\sigma}^2)}{\prod_i \Phi(y_i, \mu_i, \sigma^2)}\right)$$

QTL genotype probabilities were verified based on available data from the marker genotypes. With the H statistic, the presence of QTLs was obtained after the LOD Score estimates, which is a non-parametric statistic that follows the approximate χ^2 distribution and can be converted to the LOD = H/(2ln10) statistic. The groups are ranked according to their respective rank sums, and the H-statistic is subsequently calculated by the following estimator:

$$H = \sum_i \left(\frac{n - \sum_i P_{ij}}{n}\right)\left[\frac{\left(S_j - \mu_{0j}\right)^2}{V_{0j}}\right]$$

Where: μ_{0j} is the mean and V_{0j} is the variance of S_j in the condition of null hypothesis for

the presence of the QTL linked to the marker locus.

It was adopted as an evidence criterion of possible QTL peaks of LOD Scores with values greater than 2.5. A permutation test was performed using the scanone function by including n.perm=1000. Then, the percentage of the variance explained by the QTL was estimated through the "makeqtl" function of the R-QTL package.

Results

Quantitative analysis

The AUDPC ranged from zero to 29.5 area units. This interval was divided into six classes (HR, R, MR, MS, S and HS), based on the severity scale by Montri et al. (2009). Plants with average AUDPC less than 9.82 were considered resistant. Anthracnose symptoms were observed in all fruits of the susceptible parent (P_1). The mean values of AUDPC, IP and LP for fruit of this parent in immature stage were 15.97; 2.90 days and 5.40 days, respectively, confirming their susceptibility (Figure 2). The fruits of the resistant parent (P_2) had lower AUDPC values, with an average of 3.44, IP of five days and no apparent signs of the pathogen until the seventh day of evaluation, being considered highly resistant.

The F_1 plants showed symptoms classified as moderately resistant, also showing low AUDPC values, with an average in the F_1 population equal to 5.19. The mean of IP was intermediate (3.9 days) and the mean of LP was high (7.6 days), that is, the majority did not present a latent period in the evaluated interval, confirming the resistance (Figures 2 and 3).

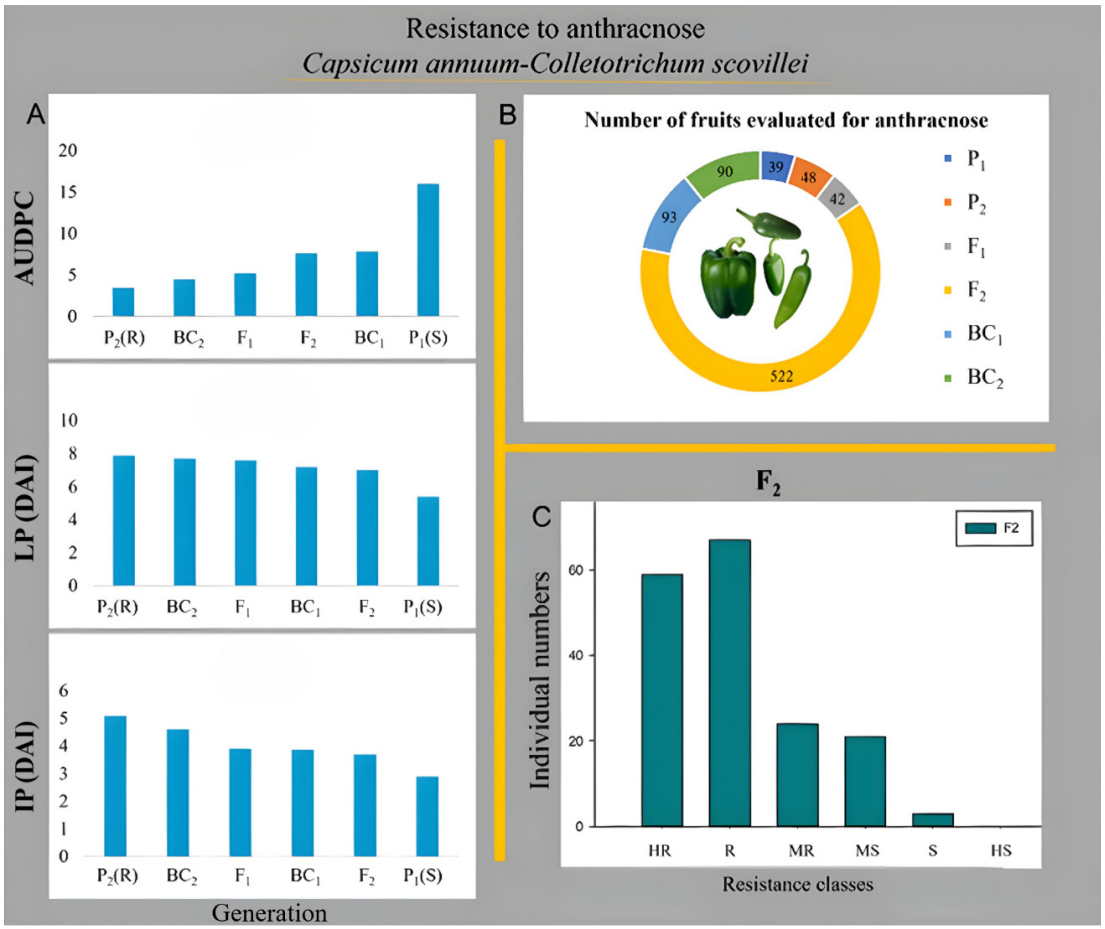

Figure 2. A) Means obtained for Area Under the Disease Progress Curve-AUDPC, Incubation Period - IP and Latent Period - LP in plants of the P_2, BC_2, F_1, BC_1 and P_1 generations from the crossing between UENF 2285 x UENF 1381 (both *Capsicum annuum*) for resistance to anthracnose caused by *Colletotrichum scovillei* in immature fruits. DAI= Days after inoculation. B) Number of fruits evaluated for anthracnose. C) Distribution of the F_2 population into six phenotypic classes related to resistance to anthracnose based on the AUDPC: HR – Highly Resistant; R – Resistant; MR – Moderately Resistant; MS – Moderately Susceptible; S – Susceptible; HS – Highly Susceptible.

The F_2 plants showed a wide variability in resistance to anthracnose, ranging from highly resistant plants with fruits without any symptoms (grade 0) to highly susceptible plants (grade 9) characterized by having fruits with more than 25% of the injured area necrotic and/or lesion around the fruit with great abundance of acervuli (Figure 3). The average of the AUDPC was also directed to the resistant parent, with a value of 7.63. Mean IP was intermediate (3.7 days) and LP was high (7 days), with a conidial mass visible to the naked eye only on the last day of assessment.

In immature fruits, in the F_2 generation (Figure 2) it was possible to observe a normal distribution of individuals between classes for resistance to anthracnose, with the curve shifted towards the resistant parent. Most F_2 individuals were grouped into Highly Resistant and Resistant classes. The segregation ratios of resistant to susceptible plants of 3:1 and 1:1 were tested for the F_2 and BC_1 generations, respectively. The segregation ratios of 3:1 in the F_2 population and 1:1 in the BC_1 population indicate that the resistance is controlled by a dominant gene, because if the deviations are not significant, it is concluded that they are due to chance, accepting the hypothesis formulated, considering that the observed values fit the expected proportion (Table 1).

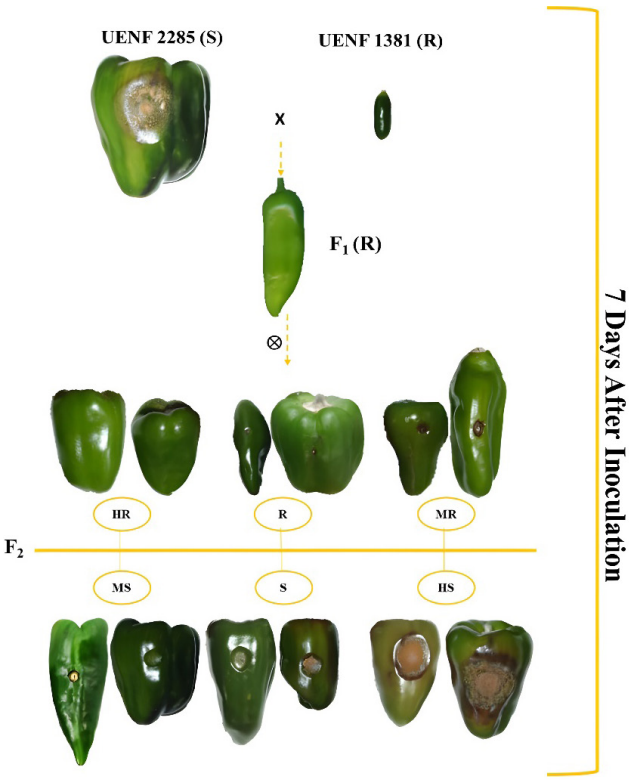

Figure 3. Reaction to anthracnose (*Colletotrichum scovillei*) and different fruit shapes and sizes in the immature stage of the parentals UENF 2285 and UENF 1381, both *Capsicum annuum* L., and of the respective F_1 and F_2 generations. Images taken seven days after inoculation. HR= Highly Resistant; R= Resistant; MR= Moderately Resistant; MS= Moderately Susceptible; S= Susceptible; S= Highly Susceptible.

Table 1. Chi-square (χ^2) test for Mendelian segregation of anthracnose (*Colletotrichum scovillei*) resistance in segregating populations of *Capsicum annuum* L.

Generation	Number of Plants			Hypothesis	X^2	*p*-value
	Total	Resistant	Susceptible			
P_1	13	0	13	0:1	-	-
P_2	16	16	0	1:0	-	-
F_1	14	13	1	1:0	-	-
F_2	174	126	48	3:1	0.621*	0.4307
BC_1	31	20	11	1:1	2.61*	0.1059
BC_2	30	27	3	1:0	-	-
X^2 tab. (1DF) = 3.84						

No significance $p \geq 0.05$.

Estimation of genetic parameters

Data obtained for AUDPC were used to estimate genetic parameters. The values of phenotypic, environmental and genotypic variances (Table 2) show that 62.75% of the phenotypic variability for AUDPC/anthracnose severity in fruits observed in the population is due to genetic factors. The genotypic variance was predominantly additive (77%) and with a smaller contribution from the domi-

nance variance (23%). The predominance of genotypic variation over environmental variance was reflected in high heritability values, with magnitudes of 62.75% and 48.47% for heritability in the broad and narrow sense, respectively.

Transgressive segregation for susceptibility in F_2 was observed, with a maximum value of AUDPC of 22.83. The average degree of dominance was 0.76, demonstrating partial dominance-type gene action. The estimate of the minimum number of genes that control resistance to anthracnose in the studied population was six, indicating a polygenic-type resistance (Table 2).

Table 2. Genetic parameters obtained from the AUDPC values to assess resistance to anthracnose in immature fruits of *Capsicum annuum* L.

Genetic parameters	Estimative
Phenotypic variance (σ_p^2)	25.42
Environmental variance (σ_e^2)	9.46
Genotypic variance (σ_g^2)	15.95
Additive variance (σ_a^2)	12.32
Dominance variance (σ_d^2)	3.62
Broad sense heritability (H^2%)	62.75
Narrow sense heritability (h^2%)	48.47
Dominance mean degree	0.76
Maximum value in F_2	22.83
Minimum value in F_2	0
Number of genes (based on variances)	6

Mean generation analysis

The genetic parameter with the highest value was the mean (15.75) followed by the additive effect (6.37). The dominance effect value was negative. The sign of the dominance component depends on the predominant direction of dominance (Mather and Jinks, 1984). The effects of mean, additivity and dominance were significant in the t-test at 1% probability. Additive-additive, additive-dominant, and dominant-dominant epistatic effects were significant in the t-test at 5% probability (Table 3).

The non-orthogonal decomposition of the sum of squares was performed in the complete model and it can be seen that the most important effect in controlling the resistance character was the additive effect, with a coefficient of determination of 72.88%, followed by the average with 15.80%, indicating the importance of these effects in controlling anthracnose resistance (Table 3).

Table 3. Estimation of genetic effects and significance test of the null hypothesis of genetic parameters (t) in the complete model and in the additive-dominant model in immature fruits of *Capsicum annuum* inoculated with *Colletotrichum scovillei*.

Complete model			
Effect	**Estimative**	**Variance**	**t**
m	15.63	7.61	5.66**
a	6.26	0.26	12.16**
d	-21.56	57.69	-2.80**
aa	-5.92	7.35	-2.18*
ad	-5.77	6.07	-2.34*
dd	11.12	26.75	2.14*
Additive-Dominant Model			
m	9.57	0.21	20.52**
a	5.87	0.21	12.73**
d	-4.51	0.86	-4.86**

** and * - Significant at 1% and 5% probability by t test, respectively.

In the non-orthogonal decomposition of the sum of squares, testing the additive-dominant model (Table 3), it was found that the most relevant parameter in controlling the resistance character was the mean. The t-test indicated significance for all parameters at 1% probability. In the additive-dominant model, the coefficient of determination for the mean was 69.39% and for the additive effect it was 26.14%. The coefficient of determination presented from the expected and observed means for the additive-dominant model was 0.97.

Selection of individuals for anthracnose resistance and fruit size

The F_2 genotypes with the lowest rank sum index values for the traits AUDPC, IP, LP, fruit length (FL) and fruit diameter (FD) were selected to advance to the next generation (Table 4). The variables AUDPC, IP, LP and FL had a selection gain of -31.59%, 10.07%, 5.75% and 2.02%, respectively, in the first selection cycle.

Table 4. Classification of F_2 individuals resistant to anthracnose (*Colletotrichum scovillei*) from *Capsicum annuum* L. var. *annuum* crosses (UENF 2285 × UENF 1381) applying a 35% selection pressure by the Mulamba and Mock Index (1978) based on resistance and fruit size variables.

Identification		Traits					Identification		Traits				
Rank	F_2	AUDPC	IP	LP	FL	FD	Rank	F_2	AUDPC	IP	LP	FL	FD
1	235	0.17	7.7	8	88.1	28.2	31	260	3.50	4.0	8.0	65.3	25.1
2	317	0.00	8.0	8	74.8	27.6	32	158	2.83	5.3	7.7	91.6	24.8
3	313	0.00	8.0	8	53.4	32.3	33	41	3.83	3.7	8.0	75.4	31.0
4	101	1.17	6.7	8	62.5	31.1	34	171	3.50	4.0	8.0	72.9	20.6
5	245	0.00	8.0	8	42.2	28.2	35	328	3.50	5.0	8.0	69.4	21.8
6	120	0.83	6.7	8	60.8	24.6	36	95	3.67	4.0	8.0	98.4	19.6
7	170	1.50	6.3	8	83.4	23.3	37	52	3.83	3.7	8.0	68.0	29.7
8	309	1.83	6.3	8	79.3	29.2	38	47	3.50	4.0	8.0	41.7	27.1
9	104	1.50	6.3	8	68.9	26.3	39	37	4.00	4.0	8.0	64.6	28.7
10	123	1.67	6.0	8	73.8	25.1	40	325	3.50	4.0	8.0	47.0	22.0
11	307	0.00	8.0	8	36.7	20.9	41	48	3.00	5.3	7.7	67.5	26.5
12	310	1.83	6.0	8	59.0	30.0	42	305	3.67	4.3	8.0	52.3	22.1
13	116	2.00	6.0	8	52.7	31.5	43	308	4.17	3.3	8.0	60.3	31.8
14	155	2.50	5.0	8	79.3	30.2	44	182	3.17	6.0	7.7	57.4	24.1
15	255	2.50	5.0	8	55.4	38.0	45	50	3.00	6.0	7.3	72.4	21.6
16	164	2.00	6.0	8	63.2	23.5	46	177	4.83	3.0	8.0	77.2	30.3
17	301	2.67	5.7	8	64.1	35.4	47	318	4.50	3.0	8.0	56.9	28.2
18	88	2.50	6.3	8	48.9	31.5	48	319	4.50	3.0	8.0	65.2	24.6
19	254	2.67	5.0	8	51.7	41.1	49	24	4.83	3.0	8.0	53.1	32.1
20	247	2.50	5.0	8	64.5	25.1	50	175	4.50	3.0	8.0	54.4	24.3
21	161	2.83	5.3	8	63.3	34.1	51	153	4.50	3.0	8.0	33.2	26.8
22	237	2.83	5.3	8	68.5	30.3	52	184	4.50	3.0	8.0	55.9	20.3
23	228	2.83	5.0	8	83.4	26.7	53	151	3.83	5.7	7.7	61.9	28.3
24	253	3.00	4.7	8	86.1	29.1	54	226	5.17	3.7	8.0	85.1	28.8
25	152	2.83	4.7	8	74.2	23.2	55	91	5.33	4.0	8.0	211.9	30.2
26	321	2.83	5.0	8	71.2	21.3	56	231	4.83	2.7	8.0	52.6	24.9
27	234	3.50	4.0	8	55.4	30.8	57	176	4.83	3.7	8.0	41.8	26.5
28	323	3.50	4.0	8	49.3	32.6	58	181	5.00	5.0	8.0	58.2	21.3
29	335	3.50	5.0	8	54.8	29.7	59	49	5.17	2.3	8.0	51.4	28.7
30	241	3.50	4.0	8	61.4	27.6	60	42	4.17	6.0	7.7	57.1	26.7

AUDPC – Area under the disease progress curve; IP – Incubation Period; LP – Latent period (days); FL – Fruit Length and FD – Fruit Diameter (mm).

QTLs identification

Six different QTLs of minor effect were identified, which together explained 23.16% of the phenotypic variation, varying from 2.45 to 7.57% (Table 5, Figure 4). Among the six QTLs, only one (QTL 2) wasn't significant. The QTL 1 presented significane in 0.01 on the F test, the QLTs 4 and 5 were significant to 0.05, and the QTLs 3 and 6 were significant to 0.1. The largest effect was displayed by QTL 1 in linkage group one; this QTL explained 7.57% of the observed phenotypic variation, with an additive effect of -0.29. It presented an LOD score of 2.98, it was positioned at 58 cM, close to the marker AFLP 45, and thus was the most important QTL identified in this study. The six identified QTLs provided an explanation of 23.16% of the phenotypic characteristics evaluated, representing important estimates of the loci position responsible for the resistance of the *C. annuum* var. *annuum* species to *C. scovillei* in unripe fruits.

Table 5. Characterization of the QTLs identified for resistance to anthracnose in unripe fruits in an F_2 population of *Capsicum annuum* var. *annuum*.

QTL	Linkage Group	Marker[1]	Position (cM)	LOD score	% of variation	Additive effect	*P* value (F)
QTL1	LG1	AFLP 45	58.0	2.98	7.57	- 0.29	0.00193 **
QTL2	LG2	AFLP 17	209.3	0.99	2.45	0.08	0.12350
QTL3	LG3	AFLP 108	98.0	1.19	2.95	0.19	0.08147 ·
QTL4	LG5	AFLP 106	86.0	1.87	4.68	- 0.04	0.01960 *
QTL5	LG9	AFLP 111	56.0	1.76	4.40	0.17	0.02449 *
QTL6	LG11	ISSR 1	51.9	1.37	3.41	- 0.01	0.05537 ·
Total					**23.16%**		

[1]Marker closest to the QTL position on the linkage map. Significance codes: ** $p < 0.01$; * $p < 0.05$; · $p < 0.1$.

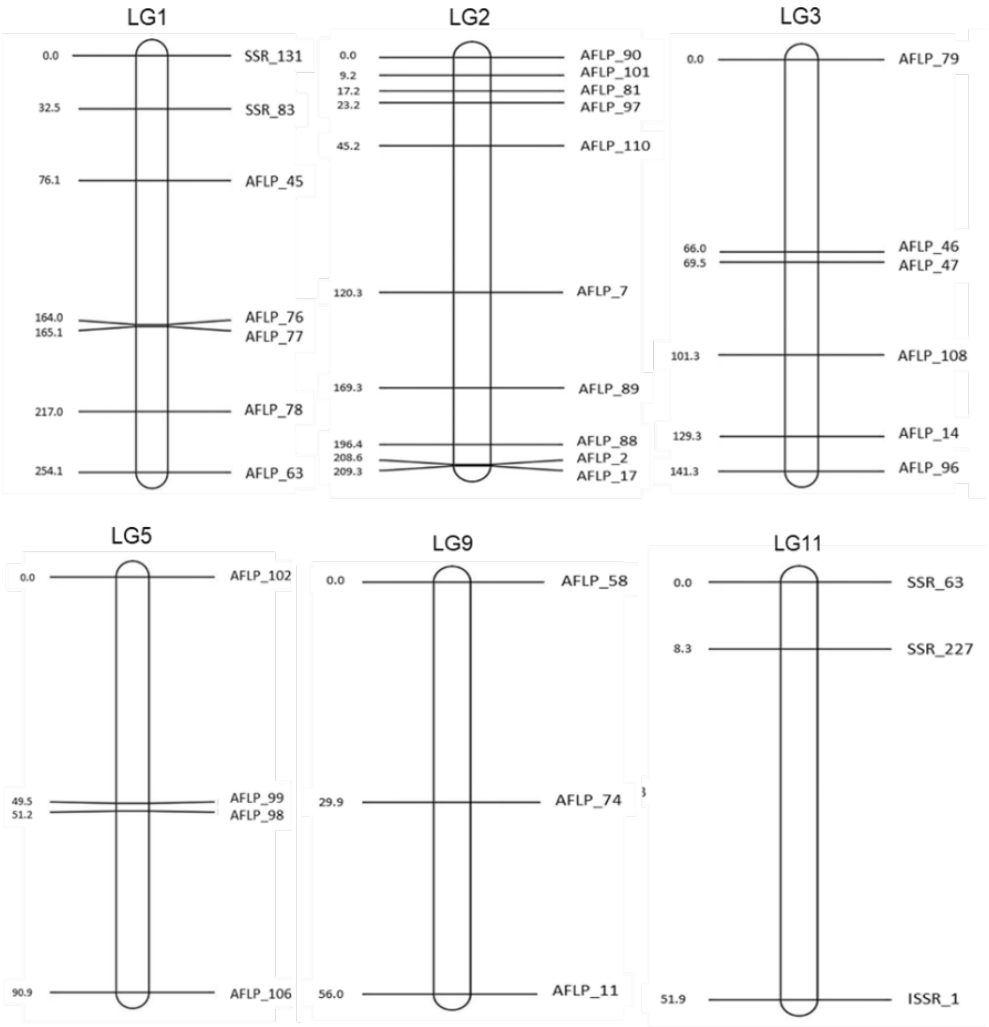

Figure 4. Linkage groups from a F_2 population of *Capsicum annuum* var. *annuum* that contains the six minor QTLs related to unripe fruits of the species. The genetic distance between each marker is indicated on the left side and the markers are indicated on the right of each group.

Discussion

The discrepancy regarding the resistance between the parents was confirmed, with the high susceptibility of parental 1 and the high resistance of parental 2, enabling the study of inheritance. The resistance of UENF 1381 (P_2), a *C. annuum* var. *annuum* accession, was previously reported (Bento et al., 2017). The stability of resistance in P_2 and the attainment of fertile F_1 and F_2 generations, allow the transfer of this resistance to intraspecific crosses in chili and sweet pepper genotypes, enabling the continuation of the *C. annuum* anthracnose breeding program.

Fruits from F_1 plants were mostly resistant to anthracnose, indicating that dominant genes control this resistance. Based on population segregation rates, this hypothesis was confirmed by the chi-square test (χ^2), estimating that a dominant gene is responsible for this resistance. According to (Kim et al., 2007), dominant resistance often justifies the production of resistant F_1 hybrids, as the obtaining of hybrids is faster than the development of inbred lines recombined with recessive genes.

Inheritance studies identified recessive genes responsible for controlling anthracnose resistance in immature fruits of *Capsicum* spp. (Pakdeevaraporn et al., 2005; Kim et al., 2007; Kim et al., 2008a; Mahasuk et al., 2009a; Mahasuk et al., 2009b; Suwor et al., 2017; Rahman and Akanda, 2022). However, for the same fruit development stage, segregation of resistance (resistant: susceptible) adequate to the Mendelian 3:1 ratio, indicating resistance control by a dominant gene, was reported only for two genotypes, line 83-168 (*C. annuum*) resistant to *C. capsici* (Lin et al., 2002; Lin et al., 2007) and PI594137 (*C. baccatum*) resistant to *C. acutatum* (Kim et al., 2008b). Two dominant genes controlling resistance in immature fruits were detected by Lin et al. (2007) on a line of *C. annuum* derived from PBC932 (*C. chinense*) resistant to *C. acutatum*.

The additive-dominant model was efficient in explaining our results, as the epistatic effects were not important in the genetic control of anthracnose resistance. The same population was tested for another pathosystem (*C. annuum – Xanthomonas euvesicatoria*) and the additive-dominant model was also sufficient to explain the genetic effects observed from the AUDPC (Silva, 2017). The similarity between the different resistances in the same genotype may be due to substances associated with resistance to fungi and bacteria that share the same metabolic route or may originate from different routes. One example is salicylic acid, which mediates different resistance responses to both fungi and bacteria, and is derived from two possible routes, isochorismate synthase (ICS) and phenylalanine ammonia-lyase (PAL) (Lefevere et al., 2020). High concentrations of caffeic and chlorogenic acid, secondary metabolites widely known to provide a defense response of plants against pathogens were observed in GBUEL 104 (= UENF 1381) on the eighth day after inoculation with *C. gloeosporioides* (Baba et al., 2019). Caffeic acid participates in the biosynthetic route of phenylpropanoids, whose precursor is PAL, and its concentration has also been related to defense against *Xanthomonas* spp. in some pathosystems (Santiago et al., 2009; Talreja and Nerurkar, 2018).

For anthracnose resistance, the additive effect had the greatest magnitude. For *C. annuum*, a species considered autogamous, this effect is important, as the frequency of homozygotes in the population tends to increase with generations, fixing resistance characteristics in subsequent generations (Lobo et al., 2005). A similar result was reported for the inheritance of resistance to *C. acutatum* in *C. annuum* in which the additive effect was the parameter with the greatest magnitude, while the dominance effect was null (Syukur et al., 2013). Plants from the F_2 generation demonstrated a wide variability of symptoms, from symptomless to highly susceptible fruit (Figure 2). However, the average trend was towards resistance (3:1), in which the majority of individuals were allocated to the Highly Resistant (HR) and Resistant (R) classes, confirming what was expected for the control of resistance governed by a dominant gene.

The heritability in the broad sense, encompassing the additive and dominance variance, was 62.75%, the highest value so far found in the consulted literature. A similar value for the variable percentage of disease incidence (h_b= 61%) and the disease index (h_b= 56%) were reported the genetic inheritance of resistance in the *C. baccatum* - *C. acutatum* pathosystem (Yoon et al., 2009). A lower value for the broad sense heritability (52%) was observed when studying the pathosystem *C. annuum* - *C. scovillei* (Giacomin et al., 2020). When analyzing the genetic parameters for resistance to anthracnose caused by *C. acutatum* in a complete diallel, using five genotypes of *C. annuum* L., observed

that the estimate of heritability in the broad sense was 47.5% (Syukur et al., 2013). The values found in this study demonstrate that the population under study has the potential for genetic gains that make it possible to continue the breeding program.

The additive variance corresponded to 77% of the genotypic variance, directly reflecting on the heritability in the narrow sense, which was 48.47%, a value considered average. Approximately 50% of the total genetic variation (H^2) observed for AUDPC in the studied population is attributed to the genetic cause of an additive nature (h^2), allowing an efficient selection of anthracnose-resistant individuals with genetic gains, including in early generations and fixation of this characteristic to the over successive generations of self-fertilization (Ramalho et al., 1993). Kim et al. (2004) reported 46.7% of heritability in the narrow sense, a value similar to that found in this work.

Transgressive segregation for susceptibility in F_2 was observed, with a maximum value of AUDPC equal to 22.83. This value exceeded the maximum values observed for the UENF 2285, the susceptible parent, showing that more than one gene controls resistance to *C. scovillei*. The transgression cannot be detected towards the resistant parent, as the threshold for resistance is the zero value. Four individuals (245, 307, 313 and 317) had zero value of AUDPC with respective IP and LP of eight days equal to the resistant parent.

The gene action observed was partial dominance (DMD = 0.76). This same type of dominance was estimated when analyzing resistance to *C. acutatum* in a complete diallel with *C. annuum* genotypes (Syukur et al., 2013), and in immature fruits (DMD = 0.66) of *C. annuum* resistant to *C. scovillei* (Giacomin et al., 2020). The value of the dominance effect was negative, and since the AUDPC is a variable for evaluating the resistance disease, lowest values are the desirable ones.

From quantitative analysis, the estimate of the minimum number of genes that control resistance to anthracnose in the studied population was six, indicating a polygenic resistance. However, in the qualitative analysis, the segregation in F_2 was adjusted to a 3:1 ratio, indicating monogenic control. The most likely hypothesis is that the control of resistance is due to the existence of a larger gene with a dominant effect associated with genes with a smaller effect. These results confirm other data available in the literature that indicate the inheritance of resistance to anthracnose in *Capsicum* as a complex trait, making obtaining resistant cultivars more challenging for plant breeders. For example, the control of resistance of the PBC932 genotype to *C. acutatum* was determined to be recessive monogenic in the HR (BC_3F_6) derived line of PBC932 (Kim et al., 2007). In other studies, resistance control identified in 0038-9151 line was attributed to two complementary dominant genes (Suwor et al., 2017) and a QTL with a major effect on chromosome P5 and four QTLs with minor effect on chromosomes P3, P7, P10 and P12 (Sun et al., 2015). This same population was re-sequenced (fine mapping) and refining the map it was possible to identify five genes in the *AnRGO5* QTL, including the *CA05g17730* gene. This gene encodes a putative R1C-3-like late blight resistance protein produced as a reaction to the oomycete *Phytophthora infestans* (Zhao et al., 2020).

Using three anthracnose resistance variables (AUDPC, IP, LP) and fruit length and diameter, 60 genotypes were selected according to Mulamba and Mock (1978). All selected genotypes were resistant to anthracnose and had fruits with an average of 28 and 63 mm in diameter and length, respectively. Low values of AUDPC and high values of LP characterized highly resistant genotypes, only the IP had values lower than expected. The genotypes with the lowest IP had no evolution of symptoms, with the value of 5.33 area units having been recorded as the highest AUDPC for these genotypes, a value close to the resistant parent (3.44). Plants with fruits whose AUDPC was less than 9.82 were considered resistant. Most plants had no results observed for the latent period within the number of days evaluated, and only six genotypes showed signs on the seventh day of evaluation. Some fruits had only

a black necrotic spot at the inoculation site and had no lesion progress, similar to a hypersensitive response, as reported by Talreja and Nerurkar (2018). These authors evaluated compatible and incompatible interactions through microscopic analysis in the PBC80 genotype (*C. baccatum*) and observed programmed cell death at the inoculation site.

The joint selection by the Mulamba and Mock index was efficient. Most variables had gain except for fruit diameter. Although the gain was nil for this characteristic, it was included due to its importance in the selection in order to obtain fruits with sweet pepper sizes and shapes. The Mulamba and Mock (1978) was already used in *Capsicum* (Medeiros et al., 2018) in the selection of *Capsicum* genotypes aiming resistance to PepYMV and agronomic characters and in the selection of genotypes with larger mass and ascorbic acid content (Medeiros et al., 2018). Luz et al. (2018) compared four selection indices for 11 morphoagronomic traits of *Capsicum* and the index proposed by Mulamba and Mock (1978) resulted in the greatest genetic gain.

The number of QTLs related to anthracnose resistance and their positions have varied among studies according to the mapping populations used, disease evaluation methods, and inoculum concentration. In this study, six minor QTLs were identified for resistance to anthracnose in the unripe fruit stage in six different linkage groups: LG1, LG2, LG3, LG5, LG9 and LG11. While it was identified a minimum number of six genes that control the resistance at this population, and the additive-dominant model was sufficient to explain the results obtained.

According to our results, 62.75% of the variation observed in the population for resistance to anthracnose is due to genetic causes. The genotypic variance was predominantly additive (77%). For *Capsicum*, this effect expresses great importance, as homozygous plants will be more abundant in the population and the additive genetic effects indicate that the trait will be fixed in subsequent generations (Lobo et al., 2005). The QTL analyses further indicated that most of the genetic variation was explained by a QTL in linkage group one (7.57%), with an LOD of 2.98. This is a promising identified locus, responsible for explaining the greatest part of the phenotypic variation in this work and presenting the well-analyzed LOD score.

A minor QTL located in LG2 at 209.3 cM, close to the marker AFLP 17, was identified; it was responsible for explaining 2.42% of the phenotypic variation and had an additive effect of 0.08. While studying QTLs for resistance to anthracnose in two *Capsicum* sources, Mahasuk et al. (2016) found two QTLs corresponding to the resistances to anthracnose in mature green and ripe fruit maturity stages, at the same location of the LG2 (56.9 cM). The LOD values of the QTLs were 3.25 and 4.21, with ability to explain total phenotypic variation of 19.5 and 18.2% and additive effects of 0.52 and 1.68, respectively.

An interspecific map between *C. annuum* and *C. chinense* identified QTLs for anthracnose resistance in linkage groups 3 and 5 (Sun et al. 2015). Different from the QTLs identified in this work in the LG3 and LG5, those authors found a QTL located at 41.8 cM for LG3 with an LOD of 2.3 that explained 2.93 of the resistance at the mature fruit stage. For LG5, six QTLs were found, located between 0.0 cM and 1.6 cM, with an LOD score ranging from 2.65 to 32.26, explaining between 9.31 and 62.38% of the variation for resistance in mature stage fruits (Sun et al., 2015). Kethom and Mongkolporn (2021) found QTLs at the LG 8 and LG 3 associated with resistance to anthracnose at mature green stage fruit in *C. baccatum* 'PBC-80'-derived recombinant inbred lines, located between SNP541 and SNP571 with 1.28 cM apart at the LOD score 5.21 and additive effect of 4.96, and a minor QTL located between SNP228 and SNP218 with 0.34 cM apart, with LOD score of 3.46 and additive effect of -4.05, respectively.

We could not compare the exact physical positions of the QTLs found here with those detected in the different studies due to the absent number of common markers and the lack of sequence information. Nevertheless, the number of QTLs, the predominance of the additive variance and the minimum num-

ber of genes that control the resistance to anthracnose in unripe fruits found in this study is a promisor indicative of the association between the phenotypic information and the quantitative loci that control the trait.

Conclusions

The study of genetic inheritance made it possible to estimate that resistance to anthracnose in fruits at the immature stage in the evaluated population is governed by at least six genes, with a dominant gene with major effect and other genes with minor effect, with a predominance of additive gene effect. The high heritability of resistance to anthracnose allows this character to be fixed in subsequent generations of selection. Six QTLs with minor effects, related to anthracnose resistance in unripe pepper fruits, were identified in six different linkage groups. Together they explained 23.16% of the phenotypic variation for the trait. The joint selection of resistance characteristics and fruit size allowed selecting 60 anthracnose-resistant *C. annuum* genotypes to *C. scovillei* with fruit length and diameter that tend to favor the development of anthracnose-resistant pepper cultivars.

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