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# Inheritance of bacterial spot disease in *Capsicum annuum* L.

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**ABSTRACT -** *Bacterial spot caused by* Xanthomonas axonopodis *pv.* vesicatoria *is considered one of the most destructive diseases in sweet pepper. Long rain periods, low resistance of the national cultivars, and inefficient chemical control favor infection and disease development. Inheritance of resistance to bacterial spot (BS) was investigated with $P_1$, $P_2$, $F_1$, $F_2$, $BC_1$, and $BC_2$ generations obtained from the cross 'Hercules' ($P_1$) x UENF 1381 ($P_2$). All these generations (40 $P_1$ plants, 40 $P_2$ plants, 40 $F_1$ hybrids, 268 $F_2$ plants, 80 $BC_1$ and 80 $BC_2$ plants) were cultivated in a greenhouse. These plants were inoculated with isolate ENA 4135 ($10^3$ cells $mL^{-1}$ per 1.0 $cm^2$ mesophile) to assess their reaction to bacterial spot. Broad- and narrow-sense heritabilities were 82.54 % and 50.17%, respectively. The average dominance degree indicated presence of overdominance and at least three recessive genes control resistance inheritance.*

**Key words**: *Capsicum annuum* L., *Xanthomonas axonopodis* pv. *vesicatoria*, disease resistance, heritability, genetic parameters.

## INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is a very important vegetable in Brazil, but its yield is affected by numerous limiting factors (Maringoni and Kimati 1987), including diseases. Bacterial spot caused by *Xanthomonas axonopodis* pv. *vesicatoria* bacteria (Vauterin et al. 1995) is considered to be one of the most destructive diseases in sweet pepper (Kimura 1984). Disease infection and development are favored by long rain periods, low resistance of national cultivars, and inefficient chemical disease control, often by antibiotics that favor the appearance of drug-resistant strains (Aguiar et al. 2000). The disease occurs at any stage of the sweet pepper development, but is most detrimental to seedlings at the greenhouse stage and to canopy organs of the adult plant, especially leaves, stems, and fruits, which present typical necrotic symptoms (Kimura and Carmo 1996).

Several studies on resistance to bacterial spot in sweet pepper have been carried out. A hypersensitive resistance response (Stall and Cook 1996) controlled by a single dominant gene (Cook and Stall 1963) was detected in a cross with PI 163192 sweet pepper germplasm.

In a selected plant of the Japanese "Santaka" chili pepper (*Capsicum annuum* L.) variety, which produces small and pungent fruits, Ribeiro et al. (1982) detected a high level of recessive genetic resistance, and the $F_1$ generation was formed

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by susceptible individuals against *Xanthomonas campestris* pv *vesicatoria*. Results from these authors further indicated that there were distinct levels of resistance, which allowed a ranking of the lines in highly resistant, resistant, and moderately resistant. This suggests that more than one locus is involved in the trait control, but there were insufficient data to ascertain the specific inheritance type. Recently, Jones et al. (2002) identified two recessive resistance-determining genes, designated *bs5* and *bs6*, when studying the inheritance of resistance to bacterial spot.

Costa et al. (2002) indicated the accession UENF 1381 and the 'Hercules' x UENF 1381 hybrid as a superior regarding bacterial spot resistance and fruit pungency. These same authors used the analysis of Griffing (1956) to report that only additive effects were involved in the genetic control of resistance to bacterial spot in leaves and fruits, suggesting the possibility of resistance fixation in advanced generations.

The assessment of type and magnitude of the genetic effects that control a trait is important for selection purposes and for the prediction of hybrid and segregant generation performance. Generation mean analyses to assess type and magnitude of the genetic variability available in the segregant populations allow the determination of the relative importance of the genetic effect components of the studied population means (Cruz and Regazzi 2001).

This study was carried out to investigate the genetic control and estimate the heritability of resistance to *Xanthomonas axonopodis* pv. *vesicatoria* in sweet pepper populations derived from the 'Hercules' x UENF 1381 cross.

## MATERIAL AND METHODS

The experiment was carried out in a greenhouse of the UENF/PESAGRO-RIO in Campos dos Goytacazes, Rio de Janeiro state, from October 2001 to April 2002, a favorable period for bacterial spot development.

Six generations, namely $P_1$, $P_2$, $F_1$, $F_2$, $BC_1$, and $BC_2$, derived from the 'Hercules' ($P_1$) x UENF 1381 ($P_2$) cross were assessed to estimate the type and magnitude of the available genetic variability and the relative importance of the genetic effects for leaf resistance reaction to bacterial spot. Studies of Costa et al. (2002) on resistance to bacterial spot and other agronomic characteristics, such as fruit yield, indicated this cross for selection.

The 'Hercules' parent is susceptible to bacterial spot and presents shiny dark green unripe and red ripe fruits. It has a high resistance to leaf blight and is PVY resistant. The UENF 1381 parent is a chili pepper accession of the PESAGRO-RIO, previously shown to be bacterial spot resistant (Costa et al. 2002).

The $P_1$, $P_2$, and $F_1$ generations were already available (Costa et al. 2002) while the $F_2$ generation and the back crosses ($BC_1$ and $BC_2$) had to be obtained. Natural self-pollination of the $F_1$ population, which is an autogamous plant species, brought forth seeds of the $F_2$ generation. Crosses were carried out in the early morning and late afternoon to obtain the $BC_1$ ('Hercules' x $F_1$) and $BC_2$ (UENF 1381 x $F_1$) seeds. Parents, $F_1$ hybrid, $F_2$ generation, and $BC_1$ and $BC_2$ generations were cultivated in a greenhouse being 40 $P_1$ plants, 40 $P_2$ plants, 40 $F_1$ hybrid plants, 268 $F_2$ plants, 80 $BC_1$ plants, and 80 $BC_2$ plants. The plants were randomly distributed into six rows. Spacing was 1.00 m between rows and 0.50 m between plants. The normal recommended cultural practices for the crop were carried out during the experiment (Filgueira 2000).

Sweet pepper plants were inoculated with the ENA 4135 isolate to assess their reaction to bacterial spot. The isolate, tested for pathogenicity in preliminary tests, is part of to the bacteria bank of the Rural Federal University of Rio de Janeiro. The isolate was cultivated in DYGS liquid medium under agitation for 48 hours at 28 °C. The bacterial suspension was then transferred to Petri dishes containing DYGS medium. After a 40 hours growth period in a bacteriological stove, the colonies were suspended in sterile water and their cell concentration adjusted to $10^3$ cells $mL^{-1}$ by a spectrophotometer at 600 nm wavelength (Aguiar et al. 2000).

Leaves of the mid-third of the plant canopy were inoculated 40 days after transplant by infiltration of bacterial suspension into the leaf mesophile, soaking an area of approximately 1.0 $cm^2$ (Bongiolo Neto et al. 1986). The leaves were collected, digitized with a scanner, and assessed three weeks after inoculation. The number of pustules $cm^{-2}$ in the inoculated area was counted and a score scale proposed, where x is the mean number of pustules $cm^{-2}$:

score 1 = $0 \leq x \leq 15$; score 2 = $15 < x \leq 30$; score 3 = $30 < x \leq 45$; score 4 = $45 < x \leq 60$; score 5 = $60 < x \leq 75$; score 6 = $75 < x \leq 90$; score 7 = $90 < x \leq 105$; score 8 = $105 < x \leq 120$; score 9 = $120 < x \leq 135$ and; score 10 = $x > 135$.

Analysis of variance of the reaction to bacterial spot on leaves was performed by the SAS software (SAS Institute Inc 1993). The following genetic and environmental components were estimated from the variance analysis of the system constituted by the $P_1$, $P_2$, $F_1$, $F_2$, $BC_1$, and $BC_2$ generations, following the model proposed by Cruz and Regazzi (2001): phenotypic variation ($\sigma_f^2$); environmental variance within population $F_2$ ($\sigma_{we}^2$); genotypic variance within population $F_2$ ($\sigma_g^2$); additive variance ($\sigma_a^2$); variance due to dominance deviation ($\sigma_d^2$); broad ($h_a^2$)- and narrow ($h_r^2$)- sense heritabilities; average dominance degree based on variances (ADD); minimum number of genes involved in the trait control ($\eta$); generation coefficient of genotypic determination ($h^2$). Variances of generations $P_1$, $P_2$ and $F_1$ were used to estimate the environmental variation effect on the trait expression.

## RESULTS AND DISCUSSION

The coefficients of variation were 15.45% and 12.34% for the assessment of bacterial spot resistance using the pustule number and score scale, respectively, indicating good experimental precision in both cases.

The number pustule on leaves and the score scale were used to assess the bacterial spot reaction (Table 1). Although discrepant, the variance values were similar to those reported by Oliveira et al. (2003), who studied the inheritance of resistance to PRSV-W in *Cucurbita moschata*. More susceptible, plants ($P_1$ and $F_1$) showed greater variances (663.62 and 547.51, respectively). Variances of the UENF 1381 reaction to bacterial spot were smaller than those of the Hercules cultivar in assessments by the number of leaf pustules (43.87 and 663.62, respectively) and score scale (0.16 and 2.62, respectively), indicating a greater stability in the expression of bacterial spot resistance. $P_2$, which is a chili pepper accession, presents pungency; there are reports in literature that suggest that greater pungency may be responsible for resistance to diseases and pests (Jarvis and Guthrie 1972).

Results of variance analysis indicated that reaction to bacterial spot may be assessed by either the number of pustules or score scale, as both variables allowed the same conclusions. An appropriate score scale is therefore extremely useful in the assessment and selection of the most resistant individuals simplifying the researcher's work by minimizing time and labor. This is especially true when a large number of plants are to be assessed simultaneously, since evaluating score intervals is an easier process than the counting of the total number of pustules on each leaf.

**Table 1**. Analysis of variance for reaction to bacterial spot, evaluated in plants of $P_1$, $P_2$, $F_1$, $F_2$, $BC_1$, and $BC_2$ generations obtained from the cross 'Hercules' x UENF 1381 of the *Capsicum annuum* L

| Sources of variation | df | Mean Squares | |
|---|---|---|---|
| | | Number of pustules cm$^{-2}$ | Scale of scores |
| Block | 3 | 4009.44 | 17.21 |
| Generation | 5 | 35655.82** | 135.43** |
| Error | 15 | 652.40 | 2.57 |
| Plant/generation | 426 | 1124.95 | 4.30 |
| Total | 449 | - | - |
| Within $P_1$ | 27 | 663.62 | 2.62 |
| Within $P_2$ | 32 | 43.87 | 0.16 |
| Within $F_1$ | 27 | 547.51 | 1.99 |
| Within $F_2$ | 216 | 1441.60 | 5.47 |
| Within $BC_1$ | 63 | 992.25 | 4.14 |
| Within $BC_2$ | 61 | 1167.65 | 4.29 |

** Significant by F test at a 0.01 probability level

Several diagrammatic scales available for disease severity assessment use comparison standards which express the necrotic leaf area or signs of the pathogen action (Azevedo 1997). Such scales should be practical and present an acceptable degree of accuracy for any pathosystem and evaluation purpose to allow comparisons among results obtained by different researchers (James 1971). Mello et al. (1997) tested and indicated a diagrammatic scale for the tomato - *Xanthomonas campestris* pv. *vesicatoria* pathosystem, based on the percentage of lesion leaf area, which differentiates among genotypes with different resistance degrees, making an assessment easier.

Synchrony is observed among the variances of the populations for both forms of assessment. As expected, the $F_2$, $BC_2$, and $BC_1$ generations presented larger variances (Table 1). The $F_2$ and backcross generation variances have an environmental and a genetic component since different genotypes are present, the latter resulting from the sum of the additive, dominance, and epistatic variances (Pinto 1995).

The number of pustules or scores obtained practically equal broad-sense heritability ($h^2_a$) values (82.54 and 82.58%, respectively) for the reaction to bacterial spot (Table 2), and fairly close narrow-sense heritability ($h^2_r$) values (50.17 and 45.63%, respectively). These results reveal that 82% of the total variance in the $F_2$ generation is consequence of the genetic causes and, further, that 45.50% are attributable to additive genetic effects, which can be effectively fixed during selfing. Reported heritability values for reaction to diseases were somewhat variable, as this genetic parameter depends on the population and the environment studied. Poulos et al. (1991) assessed the reaction to bacterial spot in CNPH 703, ECW 10R, Agronômico 10G, and ECW 30R sweet pepper genotypes and

**Table 2**. Estimates of the genetic parameters for reaction to bacterial spot determined by the number of pustules on leaves and scale of scores, evaluated in plants of generations $P_1$, $P_2$, $F_1$, $F_2$, $BC_1$, and $BC_2$ from the cross 'Hercules' x UENF 1381 of the *Capsicum annuum* L

| Genetic parameters | Traits | |
|---|---|---|
| | Number of pustules cm$^{-2}$ | Scale of scores |
| Phenotypic variation ($\sigma^2_f$) | 1441.60 | 5.47 |
| Environmental variance ($\sigma^2_{we}$) | 251.67 | 0.95 |
| Genotypic variance ($\sigma^2_g$) | 1189.93 | 4.51 |
| Additive variance ($\sigma^2_a$) | 723.29 | 2.49 |
| Variance of dominance ($\sigma^2_d$) | 466.63 | 2.02 |
| Broad-sense heritability ($h^2_a$) | 82.54 | 82.58 |
| Narrow-sense heritability ($h^2_r$) | 50.17 | 45.63 |
| Average degree of dominance (ADD) | 1.13 | 1.27 |
| Minimum number of genes ($\eta$) | 3 | 3 |
| Generation coefficient of genotypic determination ($H^2$) | 98.17 | 89.10 |

found low narrow-sense heritability values for the number of lesions (0.0% for group 2 of the bacteria and 26.0% for group 4); lesion diameter (43.00% for both groups); and total area with lesions (31.0% for group 2 and 33.0% for group 4).

We point out that heritability is a property not only of the trait, but also of the population and the environment to which individuals are subjected. Variation and uniformity of these conditions reduce or increase, respectively, the heritability and, therefore, these heritability values refer to a given population, under particular conditions (Ramalho et al. 1993).

The average dominance degree (ADD) based on variances, which allows analyses of magnitude and direction of the deviations, was 1.13 and 1.27 for pustule numbers and scores, respectively (Table 2), showing overdominance for susceptibility (Cruz and Regazzi 2001). The coefficients of genotypic determination for reaction to bacterial spot of the generations (Table 2), which indicate how much the variability depends on genetic causes, were high in the case of the pustule number (98.17%) and score scale (89.10%) assessments.

Three genes were estimated as the minimum number of genes controlling resistance to bacterial spot (Table 2). This value was found in both the assessment pustule numbers and score scale, showing that either one of the described methods evaluated resistance consistently. The expression of resistance controlled by three genes was stable as indicated by the variance of the resistant parent (Table 1). Intermediate levels of resistance controlled by several genes are normally presumed to be more stable (Bongiolo Neto et al. 1986). Ribeiro et al. (1982) suggested that more than one gene was involved in the control of reaction to bacterial spot in a cross of the Avelar cultivar with the 'Santaka' chili pepper cultivar. Adamson and Sowell Júnior (1983) confirmed this result and further suggested that another dominant gene was responsible for the resistance of PI 322719. In PI 163189, the authors suggested that two genes were involved in the resistance control, concluding that there are different genes for resistance in the *Capsicum – Xanthomonas axonopodis* pv. *vesicatoria* pathosystem. These authors had already detected the joint occurrence of additive and dominance gene effects. Resistance was recessive in PI 271322, where a reduced number of leaf lesions was formed after artificial inoculation (Sowell Júnior and Dempsey 1977). Sousa and Maluf (2003) studied genetic parameters in a sweet pepper (*Capsicum chinense*) diallel and suggested that recessive alleles in one or few gene loci control bacterial spot resistance.

The obtained information has amplified knowledge on the control of bacterial spot resistance and is fundamental to the UENF's *Capsicum* breeding program since it ensures a safer choice of the best and most efficient breeding method. To continue the program, this population should be subjected to the pedigree method, which is the most common in autogamous species (Allard 1971).

## CONCLUSIONS

- Three recessive genes control the resistance to bacterial spot in the 'Hercules' x UENF 1381 population.
- Narrow-sense heritability for bacterial spot resistance was 50.17%, which expresses a potential for trait fixation in later generations.
- The magnitude of the interactions among the alleles of the resistance controlling genes indicates overdominance.

# Herança da mancha bacteriana em *Capsicum annuum* L.

**RESUMO -** *A mancha bacteriana causada por* Xanthomonas axonopodis *pv.* vesicatoria *é uma das doenças mais destrutivas da cultura do pimentão. Longos períodos de chuvas, baixa resistência das cultivares nacionais e ineficiência de antibióticos usados no controle favorecem a infecção e o seu desenvolvimento. As gerações $P_1$, $P_2$, $F_1$, $F_2$, $RC_1$ e $RC_2$ obtidas do cruzamento 'Hércules' ($P_1$) x UENF 1381 ($P_2$) foram utilizadas no estudo da herança da resistência à mancha bacteriana. Essas gerações (40 plantas do $P_1$ e 40 do $P_2$, 40 híbridos $F_1$, 268 plantas $F_2$, 80 plantas $RC_1$ e 80 plantas $RC_2$) foram cultivadas em casa de vegetação. Para se avaliar a reação à doença as plantas foram inoculadas com o isolado ENA 4135 ($10^3$ células $mL^{-1}$ por 1 $cm^2$ do mesófilo). As herdabilidades no sentido amplo e restrito alcançaram 82,54% e 50,17%, respectivamente. O grau médio de dominância indicou sobredominância e a resistência à mancha bacteriana foi controlada por no mínimo três genes.*

**Palavras-chave**: *Capsicum annuum* L., *Xanthomonas axonopodis* pv. *vesicatoria*, resistência a doença, herdabilidade, parâmetros genéticos.

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