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Genetic parameters and selection for resistance to bacterial spot in recombinant F_6 lines of *Capsicum annuum*

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ABSTRACT - *This study aimed to advance generations and select superior sweet pepper genotypes with resistance to bacterial spot, using the breeding method Single Seed Descent (SSD) based on the segregating population derived from the cross between* Capsicum annuum *L. UENF 1421 (susceptible, non-pungent) and UENF 1381 (resistant, pungent). The segregating F_3 generation was grown in pots in a greenhouse until the F_5 generation. The F_6 generation was grown in field conditions. The reaction to bacterial spot was evaluated by inoculation with isolate ENA 4135 of* Xanthomonas campestris pv. vesicatoria, *based on a score scale and by calculating the area under the disease progress curve (AUDPC). The presence or absence of capsaicin was also assessed. Eighteen F_6 lines were bacterial leaf spot-resistant. Since no capsaicin was detected in the F_6 lines 032, 316, 399, 434, and 517, these will be used in the next steps of the sweet pepper breeding program.*

Key words: single seed descent, genetic disease resistance, sweet pepper, *Xanthomonas campestris* pv. *vesicatoria*, AUDPC, capsaicin.

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) has a high commercial value and it is one of the 10 most consumed vegetables in Brazil (Filgueira 2005). This crop is also of particular importance among the solanaceous species cultivated in Rio de Janeiro, Brazil (Cesar et al. 2007). The economic importance of sweet pepper in Brazil and in several other countries is rising, with increasing consumption of the raw fruit and also in processed form, e.g., as dressings, seasonings or canned (Azevedo et al. 2005). Considering nutritional values, sweet pepper is the vegetable with highest vitamin C contents (up to 180 mg in 100g sweet pepper) exceeding levels in the traditional sources of this vitamin, such as citrus fruits (Lúcio et al. 2003).

There are some *C. annuum* L. varieties that produce secondary metabolites in the fruit placenta

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such as capsaicinoids, including capsaicin that determines the characteristic pungency (also hotness or piquancy) of these fruits (Harvell and Bosland 1997). In *C. annuum* L. breeding programs and specifically for sweet pepper breeding, the collection of data on pungency is fundamental for genotype selection (Jarret et al. 2003). Breeders have endeavored to develop completely "sweet" varieties, i.e., without pungency. Nevertheless, when a breeder is working with hundreds or even thousands of plants in a selection procedure, it may be laborious and rather difficult to distinguish pungent from completely pungency-free sweet pepper plants, in spite of the many technical procedures available, such as HPLC (High Pressure Liquid Cromatography) or spectrophotometer techniques, which measure the fruit capsaicinoid content with high precision. The main limitation to the use of these techniques is their high cost and labor demand. On the other hand, there is a lack in research on cultivation conditions that influence the degree of pungency (Minamiyama et al. 2005) although it is well known that the pungency degree is controlled by several genes.

Despite all the progress in the management of this crop with the inclusion of new technologies in growing sweet pepper under field and greenhouse conditions, phytosanitary problems are still a barrier for sweet pepper production and a potential risk of loss for producers (Azevedo et al. 2005), representing a research challenge.

One of the most destructive sweet pepper diseases is bacterial spot, caused by the bacterium *Xanthomonas campestris* pv. *vesicatoria* (new classification proposed: *Xanthomonas euvesicatoria*, Jones et al. 2004), which is responsible for significant losses in sweet pepper. Infection and disease development are favored by high humidity, the low resistance of national cultivars and inefficiency of chemical control, often based on antibiotics, which leads to the surge of resistant races (Aguiar et al., 2000). Bacterial spot occurs at all development stages of sweet pepper, but is most damaging to greenhouse seedlings and affects leaves, branches and fruits of adult plants, with typical necrosis symptoms (Kimura and Carmo 1996).

Among the recommended control methods, the use of resistant cultivars is one of the most efficient, due to the low costs for producers besides an enhanced quality of the final consumer product and the reduction of environmental pollution by a reduced use of pesticides.

For sweet pepper, the number of disease-resistant cultivars available is lower than desirable, and therefore more attention should be paid to the development of such lines. A new cultivar, considered resistant, must however be as good as the others on the market in the absence of the disease; otherwise it will not be accepted, independent of the resistance level (Reifschneider and Lopes 1998).

Studies on resistance to bacterial spot in sweet pepper, involving knowledge on genetic control, the identification of resistance sources and search for resistant cultivars have been carried out abroad and in Brazil (Cook and Stall 1963, Stall and Cook 1966, Costa et al. 2002, Riva et al. 2004; Juhász et al. 2006, Riva-Souza et al. 2007).

In breeding programs aimed at identifying, accumulating and fixing favorable genes, the manipulation of quantitative traits by inbreeding, crossbreeding and/or selection is essential to obtain estimates of genetic parameters. By these estimates, the nature of the action of genes involved in quantitative trait control can be identified and the effectiveness of different strategies to improve genetic gains to establish and maintain an adequate genetic base can be evaluated (Cruz and Carneiro 2003).

This information together with the choice of the ideal method are key aspects of sweet pepper breeding programs. Several methods of parent choice and developing segregating generations are available, including the so-called Single Seed Descent (SSD) method, described by Brim (1966). By this method, a segregating population can be advanced in environments that are not representative of commercial conditions. The main feature of the SSD is that less time is required to obtain homozygous lines, since the processes of genotype evaluation and selection only begins after reaching the lines in homozygosis and several generations can therefore be conducted per year (Borém 2001). Another advantage of this method is that only a small area is required to raise the segregating populations (Ramalho et al. 1993), e.g., greenhouses. It is emphasized that the SSD is also an efficient method for traits of low heritability, provided that a broad genetic base is maintained as the generations are advanced (Melo 1989).

In the *Capsicum* breeding program of Embrapa Hortaliças, the SSD method was used to advance the

generations and select genotypes for resistance to *Phytophthora capsici*. Cardoso (2007) indicated SSD as an efficient method in genetic improvement of zucchini (*Cucurbita pepo* L.) and pumpkin (*Cucurbita moschata*) since evaluation and selection of progenies of each growing season are not required until the lines are practically homozygous. Raposo et al. (2000) compared methods of developing common bean segregating populations and concluded that the SSD is most ideal for breeders, in view of the ease and flexibility of handling. Soybean (Almeida et al. 2001, Sediyama et al. 2001) and common bean cultivars (Riede et al. 2002) were developed using the SSD method.

This study aimed to develop advanced generations, estimate genetic parameters and to select sweet pepper genotypes resistant to bacterial spot by the Single Seed Descent (SSD) method beginning with the segregating population derived from a cross between *Capsicum annuum* L. UENF 1421 (susceptible to bacterial spot) and UENF 1381 (bacterial spot-resistant).

MATERIAL AND METHODS

The genotypes used in this study consisted of the F_6 generation derived from the cross UENF 1421 x UENF 1381 performed from November 2001 to April 2002 (Riva et al. 2004). Accession UENF 1421 (not pungent) is susceptible to bacterial spot and has characteristics of fruit production and quality that meet market standards. Accession UENF 1381 (pungent) is a *C. annuum* pepper and has been used as resistance source to bacterial spot in a sweet pepper breeding program of the Centro de Ciências e Tecnologias Agropecuárias (CCTA) of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) (Costa et al. 2002, Riva et al. 2004) . The generation sequence was developed from F_2 by the Single Seed Descent (SSD) method (Brim 1966, Bueno et al. 2001, Borém 2001).

The experiments were performed in a research community area of the UENF and the Empresa de Pesquisa Agropecuária do Estado do Rio de Janeiro – PESAGRO-RIO / Estação Experimental de Campos dos Goytacazes, from February 2003 to November 2005.

The F_3 segregating generation with a total of 223 plants was grown in pots in a greenhouse until generation F_5 and F_6 generation was grown in field conditions. Three seeds per cell were sown on substrate for vegetables (Plantmax®) in polystyrene trays of 128 cells. The germination percentage was 85%. In the F_3 generation, yellow fruits were observed on a plant of line 118, indicating variability in fruit color. Due to the variability for the fruit in some plants, it seemed appropriate to evaluate the two plants of the pot separately, in which variability was observed (Plant 1 and Plant 2) in the following generations. The F_5 generation comprised a total of 265 plants of which 79 plants with good germination rate and unaltered fruit shape were chosen for the F_6 generation, since the number of plants was higher than the experimental conditions in the field would allow, considering that sweet pepper is a very demanding crop in terms of management and also considering the amount of data to be recorded, including the evaluation of individual plants for bacterial spot and capsaicin presence. The unused seeds in the experiment were stored in the *Capsicum* germplasm collection of UENF.

The F_6 generation was conducted in field conditions from April to November 2005, at a plant spacing of 0.8 m between rows and 0.5 m within rows. The experiment was arranged in a randomized complete block design with additional controls, with three replications. Each block consisted of 79 F_6 lines and two controls, represented by the parents UENF 1421 and UENF 1381, with five plants per row, totaling 1,215 plants in an area of approximately 500 m^2. One row was considered border and the cultural treatments during cultivation were those recommended for the crop (Filgueira 2005).

The copper-resistant isolate ENA 4135 (Aguiar et al. 2000) was used to evaluate the reaction to bacterial spot, characterized as race T1P3 in previous tests using differentiating genotypes proposed by Jones et al. (1998). The isolate preserved in DYGS medium + mineral oil was recovered by cultivation in liquid DYGS under agitation for a period of 36 hours at 28 °C (Rodrigues Neto et al. 1986). Thereafter, the bacterial suspension was transferred with a Drigalsky loop to Petri dishes containing DYGS medium. After 36 hours of bacterial growth in the incubator, at 28 ºC, the bacterial colonies were suspended in sterile water and the concentration adjusted to 10^8 ufc μL^{-1} using a spectrophotometer. One microliter (µL) was withdrawn and added to 100 mL of distilled water, adjusting the concentration to 10^5 ufc μL^{-1}. The plants were inoculated 56 days after transplanting, by the method of infiltration of bacterial

suspension into the mesophyll (Bongiolo Neto et al. 1986, Costa et al. 2002, Riva et al. 2004).

The leaves were evaluated by seven observations, beginning on the fifth day after inoculation, at intervals of 24 hours, grading the symptoms at the site of inoculation on a 1-5 score scale, namely: 1 and 2, resistant and 3 to 5, susceptible, considering grade two as point of truncation, since most plants of the susceptible parent UENF 1421 were graded higher and the resistant parent, UENF 1381, had plants with grade two only. Grade 1 was given when there was no visible symptom; grade 2 when the color of the site was white or yellow; grade 3 was applied to leaves with more defined yellow color or some points of necrosis, grade 4 to larger necrotic spots than in grade 3 and grade 5 was given when leaves were completely died off at the inoculation site.

Subsequently, the grade values were used to calculate the area under the disease progress curve (AUDPC), using software AVACPD (Torres and Ventura 1991). Seven evaluations were performed in 17 days and all data were included to compute AUDPC.

The presence or absence of capsaicin was evaluated by immersing a portion of the placenta (about $1cm^2$) from immature fruits in a 3mL solution of ammonium vanadate. After 30 minutes, the brown / black color of the flesh indicated either the presence of capsaicin or an unaltered color, the absence of capsaicin. For the preparation of this solution, 1.0 g ammonium vanadate was dissolved in 100 mL distilled water with 15 mL hydrochloric acid. Three fruits per plant were evaluated (Derera 2000). Statistical analyses were performed using the software Genes (Cruz 2006), considering the treatments as fixed, because the lines for the F_6 generation were selected.

RESULTS AND DISCUSSION

Estimates of genetic parameters are given in Table 1. It was found that the largest fraction of phenotypic variance (9.65) was due to genetic variability (6.70), which means that most of the observed variation was of genetic nature, indicating the possibility of selection progress.

This variability can be evidenced by the mean values of AUDPC observed for reaction to bacterial spot. The mean for the F_6 lines was 17.15, whereas for the susceptible parent (UENF 1421), the value reached 26.00, and the parent considered resistant (UENF 1381), remained at 15.67. The value of line 093 was also a higher value (26.70) than that of UENF 1421 and 28 F_6 lines were more resistant than UENF 1381, including line 316, with a value of 12.25 for AUDPC.

The estimate of the genotypic determination coefficient (H^2), given by the ratio between the genotypic and the phenotypic variance, was high, nearly 70.0%, demonstrating the possibility of success with selection for bacterial spot-resistance in this population.

The H^2 parameter may vary considerably according to the unit of assessment used (evaluation of the plant, line mean, plot mean, among others), and can be influenced by the genotype-environment interaction. The estimates obtained in different experimental situations should therefore be compared based on a careful assessment of the material and methods used (Gomes et al. 2004).

Riva et al. (2004) identified narrow-sense heritability at the plant level of 50.17% when studying the inheritance of bacterial spot resistance in sweet pepper in the F_2 generation of cross UENF 1421 x UENF 1381, the same cross used in this study.

The estimation of intraclass correlation, which measures the joint effect among plants of the same line, was 43.12% for AUDPC. The magnitude of this estimate, similarly to the heritability estimate, also confirms the possibility of selection in F_6 lines. According to Bland and Altman (1990), an intraclass correlation of 0.25 to 0.5 can be considered moderate.

Table 1. Estimates of genetic parameters for reaction to bacterial spot, based on the area under the disease progress curve (AUDPC) in F_6 lines derived from the cross UENF 1421 x 1381 UENF

PARAMETERS[1\]	AUDPC
$\hat{\sigma}_f^2$	9.65
$\hat{\sigma}_\varepsilon^2$	2.95
$\hat{\theta}_G$	6.70
H^2	69.46
$\hat{\rho}$	43.12
CV_g	15.09
CV_g / CV	0.87

[1\] $\hat{\sigma}_f^2$ = Genotypic variation (mean); $\hat{\sigma}_\varepsilon^2$ = Environmental variance (mean); $\hat{\theta}_G$ = Square component that expresses the mean genotypic variability; H^2 = Coefficient of genotypic determination (Selection unit: line mean in %); $\hat{\rho}$ = Intraclass correlation (Selection unit: plot in %); CV_g = Coefficient of genetic variation (%); CV_g / CV = Index of variation

The genetic variation coefficient (CVg) was 15.09%. The ratio of CVg by CV (variation coefficient), which results in the index of variation (IV), was close to unit, 0.87. Cruz and Regazzi (2001) argue that high H^2 estimates and a CVg / CV ratio higher than the unit reflect a very favorable situation for selection. It is suggested that since the IV value is close to the unit and H^2 around 70% in this experiment, the chances of success by applying selection in this population are high and indicate promising results for breeding for bacterial spot resistance. Based on these results, we used two types of selection: selection among and within lines, and combined selection.

Selection among and within lines

By the selection among lines 16 bacterial spot-resistant lines were selected, which corresponds to 20% of all lines analyzed. The following lines were selected: 011, 032, 104, 114, 183, 226, 239, 251, 301, 316, 399, 434, 470, 474, 517, and 527. Considering the selection within lines, in each block, each selected line contributed with a different number of plants (Table 2). The gain for direct selection among lines, estimated according to the selection differential and heritability, was -14.03%. The negative value indicates that the selection reduced the population mean, which is desirable since it represents higher resistance. For the selection within lines the gain was -2.57%, and the total selection gain -16.60%. For tomato, another solanaceous crop, Abreu et al. (2008) found gains by selection among and within of -38.64% for the trait resistance to late blight in the F_5 generation, demonstrating the efficiency of this selection strategy.

Among the selected lines for resistance to bacterial-spot, it is emphasized that the F_6 lines 032, 251, 316, 399, 434, and 517 showed no presence of capsaicin, in other words, are not pungent, and are recommended to be maintained in the sweet pepper program for bacterial spot resistant cultivars.

Combined selection

Cruz and Carneiro (2003) mention that one of the possible criticisms to the selection among and within lines is the fact that superior plants of lines of intermediate performance, as well as plants with intermediate performance in superior lines, are not included in the recombination to the formation of the improved population. An alternative to the selection among and within is the selection based on the individual plant performance associated with the performance of its line. Individual plants are therefore not evaluated in two stages, but rather in a single one, where the selection criterion is the index based on the linear combination of the information of the plant and its relatives. Two indices can be used by this strategy: I and II. By index I, the value of a plant is considered in relation to the plot mean, and by index II, the value of a plant is considered in relation to the block mean (Cruz and Regazzi 2001).

Evaluating the reaction to bacterial spot by AUDPC and based on index I, 11 lines were selected (008, 032, 104, 301, 316, 339, 399, 434, 470, 474, 504) (Table 2). Line 339 was also pungency-free. The selection gain was -20.8% and the efficiency of the combined compared to selection among and within lines was 1.25, indicating that combined selection should be preferred to selection among and within, as described by Martins et al. (2005). However, despite the apparent superiority of combined selection, the genetic gains by selection among and within lines were also significant and should be maintained in the selection procedures.

Table 2. Selection gains in F_6 lines from the cross UENF 1421 x UENF 1381, for bacterial spot reaction evaluated based on the area under the disease progress curve (AUDPC), considering different selection procedures

Selection procedure	Selected F_6 lines	Selection gain (%)
Selection among and within lines	316(4)[a\], 301(4), 434(5), 470(4), 104(3), 474(6), 517(3), 399(3), 032(4), 226(10), 527(6), 239(5), 183(5), 114(5), 011(5), 251(4)	-16.60*
Combined selection (Index I)[1\]	008(2)[b\], 032(1), 104(1), 301(13), 316(14), 339(3), 399(1), 434(7), 470(3), 474(1), 504(2)	-20.80
Combined selection (Index II)[2\]	104(2)[b\], 301(14), 316(14), 434(12), 470(6)	-21.21

[1\] The plant value is considered in relation to the plot mean
[2\] The plant value is considered in relation to the block mean
[a\] The number in brackets express the quantity of plants selected within each line, in three blocks
[b\] The number in brackets express the quantity of the selected plant in that row
* Total selection gain (gain among + gain within)

Considering the value of the individual plant mean in the block (index II), the lines selected were 104, 301, 316, 434 and 470 (Table 2). The gain for selection was -21.21% and the efficiency of combined selection for the selection among and within lines reached a value of 1.28.

Cruz and Carneiro (2003) explained that by the strategy of combined selection, higher gains can be obtained by selection, because by the selection at a single stage, which considers both the value of the plant and its relatives, inferences on the genetic value of the selection units may be more accurate.

It may be said that there is no single breeding method, by which one can achieve specific objectives. For the choice of the selection method with segregating populations, each situation should be assessed for the possibility of achieving the following objectives: acquisition of information on the inheritance of traits involved, ease of performance; time required and cost and labor savings (Borém 2001).

CONCLUSIONS

The SSD method was efficient for the selection of bacterial spot resistant lines in the F_6 generation, derived from the cross between UENF 1421 x UENF 1381. The selected lines were those identified as: 008, 011, 032, 104, 114, 183, 226, 239, 251, 301, 316, 339, 399, 434, 470, 474, 504, 517, and 527. No capsaicin was found in the F_6 lines 032, 251, 316, 399, 434, and 517, which were therefore recommended to be maintained in the sweet pepper breeding program. It is also highlighted that variability was observed in the F_6 lines for other traits such as fruit shape and color, suggesting a potential for further studies in the *Capsicum* breeding program.

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Parâmetros genéticos e seleção para resistência à mancha-bacteriana em linhas recombinantes F_6 de *Capsicum annuum*

RESUMO - *O presente trabalho objetivou avançar gerações e selecionar genótipos superiores de pimentão resistentes à mancha-bacteriana utilizando-se o método de melhoramento Single Seed Descent (SSD) a partir da população segregante derivada do cruzamento entre os acessos de* Capsicum annuum *L. UENF 1421 (suscetível, não-pungente) e UENF 1381 (resistente, pungente). A geração segregante F_3 foi cultivada em vasos em casa de vegetação até se alcançar a geração F_5. A geração F_6 foi cultivada em condições de campo. A reação à mancha-bacteriana foi avaliada por meio da inoculação com o isolado ENA 4135 de* Xanthomonas campestris pv. vesicatoria*, sendo o resultado obtido por meio de notas e do cálculo da área abaixo da curva de progresso da doença (AACPD). Avaliou-se também a presença ou ausência de capsaicina. Dezoito linhas F_6 foram resistentes à mancha-bacteriana. Nas linhas F_6 identificadas como 032, 316, 399, 434 e 517 não foi registrada a presença de capsaicina e darão seqüência ao programa de melhoramento do pimentão.*

Palavras-chave: descendentes de uma única semente, resistência genética a doenças, pimentão, *Xanthomonas campestris* pv. v*esicatoria*, AACPD, capsaicina

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