

Obtaining pepper $F_{2,3}$ lines with resistance to the bacterial spot using the pedigree method

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ABSTRACT

The economic importance of sweet pepper is rising in Brazil and abroad. Nevertheless, diseases, such as the bacterial spot (*Xanthomonas campestris* pv. *vesicatoria* or *Xanthomonas euvesicatoria*) jeopardize its yield. An efficient method for controlling the disease is the use of resistant varieties. The objective of this work was to select sweet pepper genotypes with resistance to the bacterial spot on a segregating population (*Capsicum annuum* accessions UENF 1421, susceptible, x UENF 1381, resistant), advanced using the pedigree method. Three selection procedures, "selection between and within families", "mass selection", and "combined selection" were compared. Fifty-six $F_{2,3}$ families (40 plants per family) were planted in the field, without replication. Ten plants of each genitor were used as controls in each line, between families. Plants were inoculated (isolate ENA 4135) 42 days after transplanting and leaves were collected and digitalized three weeks after. Images were analyzed to obtain the percentage of leaf tissue damaged (RMB%) and leaf damaged area (RMBcm²). Since similar results were achieved using either RMB% or RMBcm², we suggest using RMB% in further studies instead of RMBcm², which values remain close to zero. Eleven lines had promising results when selection within and among $F_{2,3}$ families was used (genetic gains = 40%). Considering mass selection, eleven individuals (three lines), were selected (genetic gains = 28%). Based on combined selection, three lines (11 individuals within the lines) were selected (genetic gains = 266%). There were three lines (105, 475, and 517) in common between selection among and within families and mass selection, and another three (111, 260, and 313), between selection among and within families and combined selection. Combined selection was 6.6 (RMB%) and 6.5 (RMBcm²) times more efficient than selection among and between families, and 9.6 (RMB%) and 9.5 (RMBcm²) times more efficient than mass selection.

Keywords: *Capsicum annuum*, *Xanthomonas campestris* pv. *vesicatoria*, plant breeding, selection within and among plants, mass selection, combined selection.

RESUMO

Uso do método genealógico na obtenção de linhas $F_{2,3}$ de pimentão resistentes à mancha-bacteriana

O pimentão apresenta crescente importância econômica no Brasil e no exterior. No entanto, algumas doenças, como a mancha-bacteriana (*Xanthomonas campestris* pv. *vesicatoria* or *Xanthomonas euvesicatoria*), representam uma ameaça às lavouras. O uso de cultivares resistentes é uma medida eficiente de controle. O presente trabalho objetivou selecionar genótipos de pimentão resistentes à mancha-bacteriana em uma população segregante (acessos de *Capsicum annuum* UENF 1421, suscetível x UENF 1381, resistente), conduzida pelo método genealógico. Foram comparados três métodos de seleção: seleção entre e dentro de famílias, seleção massal e seleção combinada. Cinquenta e seis famílias $F_{2,3}$ (40 plantas por família) foram plantadas em campo, sem repetição. Dez plantas de cada genitor, colocadas entre as famílias, foram utilizadas como controle. As plantas foram inoculadas (isolado ENA 4135) 42 dias após o transplante, sendo as folhas coletadas três semanas após e digitalizadas. As imagens foram utilizadas para obter a porcentagem de tecido foliar lesionado (RMB%) e área foliar lesionada (RMBcm²). Como foram obtidos resultados similares utilizando ambas as medidas, sugere-se a utilização de RMB%, já que os valores de RMBcm² permanecem sempre próximos a zero. Quando a seleção entre e dentro de famílias foi aplicada, onze linhas apresentaram resultados promissores (ganho genético de 40%). Considerando a seleção massal, onze indivíduos (três linhas) foram selecionados (ganho genético de 28%). Utilizando a seleção combinada, três linhas (onze indivíduos nestas linhas) foram selecionadas (ganho genético de 266%). Três linhas selecionadas (105, 475 e 517) foram comuns à seleção entre e dentro de famílias e à seleção massal e, outras três (111, 260 e 313), à seleção entre e dentro de famílias e à seleção combinada. A seleção combinada foi 6,6 (RMB%) e 6,5 (RMBcm²) vezes mais eficiente que a seleção entre e dentro de famílias e 9,6 (RMB%) e 9,5 (RMBcm²) vezes mais eficiente que a seleção massal.

Palavras-chave: *Capsicum annuum* L., *Xanthomonas campestris* pv. *vesicatoria*, melhoramento genético, seleção entre e dentro, seleção massal, seleção combinada.

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The economic importance of sweet pepper is rising in Brazil and in many countries, as its use *in natura*, as well as the use of manufactured sauces, condiments, and canned food is increasing (Azevedo *et al.*, 2005). Nevertheless, sweet pepper has its yield

jeopardized due to diseases such as the bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* or *Xanthomonas euvesicatoria*, according to the new classification suggested by Jones *et al.* (2004). The bacterial spot is one of the most severe diseases of

pepper and is responsible for significant losses. The contamination and the development of the disease are favored by long rainy periods, low level of resistance of national cultivars, and inefficient chemical control, frequently carried out using antibiotics, which

promotes the appearance of resistant strains (Aguiar *et al.*, 2000). The bacterial spot is found in all sweet pepper growing stages, but it is more harmful in the seedling stage in greenhouse, and in leaves, branches, and fruits of adult plants, when symptoms are typically necrotic (Kimura & Carmo, 1996). In sweet pepper, the occurrence of eleven races of the bacteria had already been reported (Jones *et al.*, 1995; Kousik & Ritchie, 1995; Jones *et al.*, 1998; Sahin & Miller, 1998; Kousik & Ritchie, 1999; Sahin, 2001).

Among the recommended methods of control, the use of resistant cultivars is one of the most efficient, due to the low cost it represents to farmers, the increase in the product final quality to the consumer, and the decrease in the environment pollution, as well as in the use of pesticides (Silva, 2003). Thus, there is increasing interest in developing sweet pepper cultivars resistant to this disease (Sahin & Miller, 1998).

Researches about the resistance to the bacterial spot in sweet pepper are being conducted in Brazil and abroad and deal with the unraveling of its genetic control, the identification of sources of resistance, and the search for resistant cultivars (Cook & Stall, 1963; Ribeiro *et al.*, 1982; Jones *et al.*, 2002; Juhász, 2002; Costa *et al.*, 2002b; Sudré, 2003; Riva *et al.*, 2004a; Riva-Souza *et al.*, 2005). So far, combining more than one resistant gene in the same plant seems to offer the best control of the disease (Subramanya, 1999). Genes *Bs1*, *Bs2*, and *Bs3* were found in the PI 163192 (*Capsicum annuum*), PI 260435 (*C. chacoense*), and PI 271322 (*Capsicum annuum*), respectively (Reifschneider & Lopes, 1997). Gene *Bs2* offers hypersensitive resistance to the three races considered as of wide occurrence (1, 2, and 3), as well as to race 0 (Kousik & Ritchie, 1996). Gene *Bs3* offers resistance to *X. campestris* pv. *vesicatoria* strains that express the protein of avirulence AvrBs3 (Pierre *et al.*, 2000). Another approach to search for resistance to the bacterial spot is the identification of genes associated to non-hypersensitive responses and to use them together with genes which codify hypersensitive response, such as, for

example, genes *bs5* and *bs6*, which confer resistance to race 6 (Jones *et al.*, 2002). Many researches have demonstrated the presence of recessive genes in the control of resistance to the bacterial spot (Ribeiro *et al.*, 1982; Costa *et al.*, 2002b; Riva *et al.*, 2004b).

In a plant breeding program, many methods to choose parents and to obtain segregant generations are available to autogamous species. One of these methods is the genealogical method, also known as the pedigree method (Allard, 1971; Costa *et al.*, 2002a). The essence of this method is the individual selection of plants in the segregating population, based on the isolate evaluation of each progeny. The use of progeny test increases efficiency of selection. The advantage of this method is that it allows controlling the degree of parentage between selections, discarding of inferior individuals on early generations; as well as the using the data obtained to genetic studies in which plant breeders can be trained. However, one of the disadvantages of this method is that usually it is possible to obtain only one generation a year, making it very retarding; it is labor-consuming, it demands experimental fields, and requires a qualified staff to perform selection (Borém, 2001).

The objective of this work was to select sweet pepper genotypes with resistance to the bacterial spot on a segregating population derived from the crosses between the *Capsicum annuum* L. accessions UENF 1421 (susceptible) and UENF 1381 (resistant) advanced using the pedigree method. In addition, three selection procedures, namely "selection between and within families", "mass selection" and "combined selection", were compared.

MATERIAL AND METHODS

Genotypes used in this research were the segregant generation formed by seeds $F_{2:3}$ obtained from the cross between UENF 1421 x UENF 1381. The UENF 1421 accession is non-pungent (sweet pepper); presents conic fruits of 60.0 g of weight in average, and is susceptible do the bacterial spot. UENF 1381 produces pungent fruits of 3.0 g

of weigh in average (Sudré, 2003) and is resistant to the disease. This cross is part of our sweet pepper breeding program. Therefore, estimation of some genetic parameters are already available, such as strict sense heritability (around 50.8%) and *lato sensu* heritability (around 82.5%). Resistance is controlled by three recessive genes (Riva *et al.*, 2004a). Preliminary results indicate that the resistance is non-specific.

The experiment was carried out in an area from the consortium between UENF and PESAGRO/RIO, at Campos Experimental Station, from October 2003 to July 2004. The spacing between lines was 1.0 m and, between plants inside lines, 0.8 m. Fifty-six $F_{2:3}$ families, without replication, and 40 plants per family, were planted in the field. Ten plants of genitors UENF 1421 and UENF 1381 were planted as controls in each line, between families. Borders were constituted by ten plants from the F_2 generation. Cultural practices carried applied were those recommended to the crop (Filgueira, 2000).

In order to evaluate plant reaction to the bacterial spot, we used the isolate ENA 4135, resistant to copper (Aguiar *et al.*, 2000), previously characterized as race T1P3 (Riva *et al.*, 2004b). The isolate was preserved on a DYGS culture medium + mineral oil, and it was recovered through cultivation on a liquid DYGS culture medium, under agitation for 36 hours at 28°C (Rodrigues Neto *et al.*, 1986). After that, the bacterial suspension was transferred to Petri dishes containing DYGS solid culture medium, with the help of a Drigalski spatula. After a 36-hour-growing-period on a bacteriological heater, at 28°C, bacterial colonies were suspended on sterile water and its cell concentration was adjusted to 10^3 ufc mL⁻¹, via a spectrophotometer, using wavelength of 600 nm (Aguiar *et al.*, 2000). Inoculation was performed 42 days after the seedlings had been transplanted, using the method of suspended bacteria infiltration at the mesophyll (Bongioiolo Neto *et al.*, 1986; Juhász, 2002; Costa *et al.*, 2002b; Sudré, 2003; Riva *et al.*, 2004a). Inoculated leaves were collected three weeks after inoculation and were digitalized, via scanner, on the following

day (Juhász, 2002; Riva *et al.*, 2004a). Digitalized images were analyzed using the software QUANT (Vale *et al.*, 2003) to obtain the percentage of leaf tissue damaged (RMB %) and leaf damaged area (RMBcm²), considering 1.0 cm³ of foliar area inoculated.

Statistical analysis was performed using the software GENES, as well as the estimation of genetic parameters and gains by selection. The procedures “selection between and within families”, “mass selection” and “combined selection” were employed in the selection of the best F_{2,3} families (Cruz, 2001).

RESULTS AND DISCUSSION

Considering each genitor individually, no variability to the reaction to the bacterial spot was observed and 100% of plants from the accession UENF 1421 were susceptible, while all plants from UENF 1381 were resistant, as expected. This result was verified by the lack of difference among plants of each accession to the reaction to the disease. On the contrary, in the segregant generation F_{2,3}, highly significant differences between lines were observed, at 1% probability by the F test. The existence of variability between lines indicates that new cycles of selection are possible. Genitor UENF 1421, considered susceptible, averaged 22.2 for RMB% (percentage of leaf tissue damaged) and 0.23 to RMBcm² (leaf damaged area), values highly superior to those of the resistant genitor, UENF 1381, which presented averages of 1.8 and 0.02 to respectively RMB% and RMBcm². Generation F_{2,3} presented the average of 9.76 to RMB% and 0.10 to RMBcm². These values settled it in an intermediate position between the genitors, although closer to UENF 1381, the resistant genitor.

Results indicated that the reaction to the bacterial spot can be assessed both in terms of percentage and in section injured area (cm²), since both allow reaching the same conclusions. We suggest the assessment in percentage to be used in further studies instead of the assessment in cm², which values remain close to zero. The percentage provides

Table 1. Estimates of genetic parameters of the reaction to the bacterial spot, assessed in generation F_{2,3} from the cross UENF 1421 x UENF 1381 (estimativas de parâmetros genéticos para reação à mancha-bacteriana, avaliada na geração F_{2,3} obtida do cruzamento UENF 1421 x UENF 1381). Campos de Goytacazes, UENF, 2004.

Genetic parameters ¹	RMB (%) ²	RMB (cm ²)
$\hat{\sigma}_{fc}^2$	68.9	0.0071
$\hat{\sigma}_{fd}^2$	101.1	0.0104
$\hat{\sigma}_{ae}^2$	8.9	0.0009
$\hat{\sigma}_{ad}^2$	29.3	0.0030
$\hat{\theta}_{ge}$	60.0	0.0062
$\hat{\theta}_{gd}$	71.8	0.0074
$\hat{\theta}_a$	32.1	0.0034
$\hat{\theta}_d$	111.5	0.0114
H_{ae}^2	87.1	87.30
H_{ad}^2	71.0	70.78
H_{re}^2	46.6	47.31
H_{rd}^2	15.9	16.15
H_{rt}^2	28.4	28.79

¹ $\hat{\sigma}_{fc}^2$ = phenotypic variance among families (variância fenotípica entre famílias); $\hat{\sigma}_{fd}^2$ = phenotypic variance within families (variância fenotípica dentro de famílias); $\hat{\sigma}_{ae}^2$ = environmental variance among families (variância ambiental entre famílias); $\hat{\sigma}_{ad}^2$ = environmental variance within families (variância ambiental dentro de famílias); $\hat{\theta}_{ge}$ = quadratic component expressing the genotypic variability among families (componente quadrático que expressa a variabilidade genotípica entre famílias); $\hat{\theta}_{gd}$ = quadratic component expressing the genotypic variability within families (componente quadrático que expressa a variabilidade genotípica dentro de famílias); $\hat{\theta}_a$ = quadratic component expressing the additive variability (componente quadrático que expressa a variabilidade aditiva); $\hat{\theta}_d$ = quadratic component expressing the dominance deviation (componente quadrático que expressa a variabilidade devida aos desvios de dominância); H_{ae}^2 = broad sense coefficient of genotypic determination among families (coeficiente de determinação genotípico amplo entre famílias); H_{ad}^2 = broad sense coefficient of genotypic determination within families (coeficiente de determinação genotípico amplo dentro de famílias); H_{re}^2 = narrow sense coefficient of genotypic determination among families (coeficiente de determinação genotípico restrito entre famílias); H_{rd}^2 = narrow sense coefficient of genotypic determination within families (coeficiente de determinação genotípico restrito dentro de famílias); H_{rt}^2 = Total narrow sense coefficient of genotypic determination (coeficiente de determinação genotípico restrito total); ²/RMB% = percentage of leaf tissue damaged assessed using the software QUANT (Vale *et al.*, 2003) (porcentagem de área foliar com sintomas, avaliada pelo programa QUANT, Vale *et al.*, 2003); RMBcm² = leaf damaged area assessed using QUANT (área foliar afetada pela doença em cm² avaliada pelo QUANT).

numerical values greater in importance, making estimates and interpretation of the results easier.

The phenotypic variance distributed among and within families was higher within families (Table 1). Environmental variance was also predominantly distributed within families (Table 1). The greatest estimates of genotypic variance were recorded within families (Table 1). This consideration justifies selection among and within families in early generations

of self-fertilizing plants. The estimate of the quadratic component which expresses additive variability was lower than the estimate of the quadratic component which expresses variability due to dominance deviation to both RMB% and RMBcm². The presence of deviations due to dominance can make the selection process more complex, once superior phenotypes can correspond to individuals with *loci* in heterozygosity. Nevertheless, this is not a fact of much concern, because it deals

with $F_{2,3}$ generation, in which genes in heterozygosity are still present. Abreu (2005) found estimates for dominance variance superior to additive variance on an F_5 population of tomatoes, assessed to resistance to *Phytophthora infestans*.

The estimation of the coefficient of genotypic determination (CDG) is essential to the plant breeder work, because it provides the relation of the genotypic variability that is found at the total phenotypic variance, gauging the reliability of the phenotypic value as an indicator of the genotypic value (Ramalho *et al.*, 1993). CDG was estimated both in the narrow and broad sense, considering the average of the generation (among families) and the plant within the family, as well as the total narrow sense CDG, which is based on the plant individually, disregarding the family.

CDG estimates in broad sense among families were superior to those within families for the evaluated characteristics, although the latter was also relevant, superior to 70%. High CDG estimates among and within families show that phenotypic superiority can be related to genotypic superiority, providing strong reliability in the selection process among and within families. However, it is important to perform the selection within families with much care, since CDG estimates in the narrow sense within were lower than among families for both RMB% and RMBcm². Variability within families may not be a characteristic totally related to inheritance, and the environmental variance, as well as the quadratic component, which express variability due to dominance deviation, contribute to that. Thus, according to CDG estimates, the expectation is to reach greater efficiency on the selection among the families.

The applied intensity of selection among and within the $F_{2,3}$ lines were 20%. Selection criterion was the decrease on the original averages of the characteristic reaction to the bacterial spot, revealing a gain by selection in the negative sense. Families were selected and estimates of selection gains were obtained, considering the following selection strategies: i. selection among

and within families, ii. mass selection, and iii. combined selection.

Selection among and within families

Eleven $F_{2,3}$ families were selected and, within each family, only one plant was selected. Selected families were: 105, 111, 125, 157, 226, 260, 282, 288, 313, 475, and 517. Considering percentage of leaf tissue damaged (RMB%), the estimated gain with selection among families was of 38.3% and, within families, 2.0%, summing up 40.3%. When leaf damaged area (RMBcm²) was taken into account, the gain with selection among families was of 38.9% and, within families, 2.0%, resulting in a total gain of 40.9%. The gain achieved with selection among families exceeded the gain attained with selection within families. Sedyama *et al.* (1999) suggest performing selection within families only up to the generations F_3 and F_4 , due to the fact that after these generations, families already have good uniformity due to the high frequency of *loci* in homozygosis.

Mass selection

Using this strategy, eleven individuals were selected to resistance to the bacterial-spot, two of them belonging to family 550 and one in each of the following families: 41, 105, 118, 209, 341, 379, 434, 475, and 517. Gains of selection were 27.6% to RMB% and 28.1% to RMBcm².

Combined selection

Three families were selected, 111, 260, and 313. All seven individuals belonging to family 111 were also selected, while families 260 and 313 had two selected individuals each. The estimated gain of selection was of 266.2% to RMB% and 265.5% to RMBcm².

The efficiency of combined selection in comparison to the selection among and within families was of 6.6 and 6.5 to respectively RMB% and RMBcm². When mass selection is considered, combined selection presented an efficiency of 9.6 and 9.5 respectively to RMB% and RMBcm². Sampaio *et al.* (2000) observed similar genetic gains to individual selection and combined selection in *Pinus caribaea*

pv. hondurensis regarding the character wood volume. Noda *et al.* (2003) reported that the genealogical method is being used since 1976 by INPA (National Research Institute in the Amazon), in the sweet pepper breeding program, aiming at incorporating resistance to *Xanthomonas campestris* *pv. vesicatoria*. These researchers presented results about F_{13} and F_{14} progenies from the interspecific cross between *Capsicum annuum* and *C. chinense*, named HP-12. The selected progenies presented greater resistance and yielding capacity, when compared to the susceptible control (cultivar Cascadura Ikeda), when exposed to the occurrence of the disease, allowing authors to conclude that fruit productivity is related to the level of host resistance to the pathogen. The selection performed by the genealogical method was efficient and provided consistent gains.

Selected individuals in the present work will continue in use in the breeding program, with good perspectives of reaching significant gains in the resistance to the bacterial spot. In spite of the greater magnitude of gains when the combined selection was used, we suggest advancing to the next generation to be done using the families selected in the three strategies. This will preserve the genetic variability of the population, which is still in early generations of self-fertilization.

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