Genetic analysis of resistance to bacterial spot in sweet pepper genotypes

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ABSTRACT

Bacterial spot (BS) is considered one of the most important diseases in sweet pepper cultivation and can cause great yield losses. Combining abilities for leaf and fruit resistance to BS of five sweet pepper genotypes were assessed. Analysis was performed using the diallel cross design following Method II, Model I proposed by Griffing (1956). Leaves were inoculated using the infiltration method while fruits were inoculated by perforation with a hypodermic needle. Leaf reaction to BS was assessed three weeks after inoculation by a score scale ranging from 1 (resistant) to 6 (susceptible). Fruit reaction was assessed seven days after inoculation according to a score scale ranging from 1 (resistance) to 5 (susceptible). Significant General Combining Ability (GCA) effects were detected for both leaves and fruits. Specific Combining Ability (SCA) for resistance to BS was not significant for either leaves or fruits. According to the analysis of Griffing (1956), the parents BGH 1772 and BGH 3071 contributed to the increase in leaf resistance, and BGH 1772 and UENF 1421 to fruit resistance.

KEY WORDS: *Capsicum annuum* L., *Xanthomonas axonopodis* pv. *vesicatoria*, disease resistance, general combining ability, specific combining ability, diallel analysis.

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is one of the most important vegetables in the Brazilian market due to its large scale consumption and economic and nutritional values (Rodrigues and Leal, 1991). Sweet pepper cultivation techniques are being constantly improved to meet the demands of the consumer market (Soares, 1995). Breeding studies have been carried out to adapt it to the climatic and agronomic-economic conditions of the growing countries (Popa et al., 1977 quoted by Soares, 1995), and also to introduce resistance to diseases such as viruses (Nagai, 1983), soft rot (Boiteaux and Lopes, 1993), anthracnosis (Heinz et al., 1993) and bacterial spot (Reifschneider and Lopes, 1997; Stall 1997).

Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Xcv) (proposed new classification: *Xanthomonas axonopodis* pv. v*esicatoria* –Xav, Vauterin et al., 1995), is considered the main bacterial disease of the sweet pepper culture crop. It affects the plant canopy and can cause great leaf damage under certain environmental conditions, both in field and in sheltered cultivation, causing yield and fruit quality losses (Kimura, 1984b; Santos, 1995; Kousik and Ritchie, 1996; Sahin and Miller, 1996; Lopes and Quezado-Soares, 1997). The bacteria affect the plant at any developmental stage, but symptoms are most severe at the seedling, flower bud and seed box stages (Salgado and Tokeshi, 1980).

The plant is infected through natural openings such as stomata, hydathodes and lenticels. Small lesions caused by wind, rain or insects can also allow the pathogen to enter (Kimura, 1984a). Disease is disseminated from plant to plant by rain or irrigation (Carmo et al., 1996).

Among the recommended control methods, the use of genetically resistant cultivars is the most economic and technically practical, mainly when the cost, risk of fruit contamination by pesticides and pathogen resistance to chemical products are considered. Therefore, there is a growing interest in developing sweet pepper cultivars with resistance to bacterial spot (Sahin and Miller, 1998).

Three resistance genes (*Bs1, Bs2* and *Bs3*) were identified in the PI 163192 (*Capsicum annuum*), PI 260435 (*Capsicum chacoense*) and PI 271322 (*C. annuum*) accessions, respectively. In the last accession, the *Bs1* gene and factors suggesting quantitative resistance were also detected. Studies based on these three accessions showed that the interactions involving

these genes follow the gene-to-gene hypothesis. The *Bs2* gene confers resistance to races 0, 1, 2, and 3, which are commonly found but not to races 4, 5, and 6 (Reifschneider and Lopes, 1997). Estimation of the combining ability components is important in breeding programs to select genetically divergent parents in crossing schemes, mainly when identification of promising hybrids and/or development of superior lines from these hybrids are required (Allard, 1971). Diallel is a genetic-statistical design used to estimate the relative magnitude of the genetic variance components of the traits of interest to breeders (Blank, 1997). The method of diallel analysis proposed by Griffing (1956) estimates the effects of the general and specific combining abilities (GCA and SCA) (Cruz and Regazzi, 1994).

The objective of this study was to estimate the genetic components of the reaction to bacterial spot (RBS) in sweet pepper leaves and fruits using the methodology of diallel analysis proposed by Griffing (1956).

MATERIAL AND METHODS

Parents and their F1 generations of a complete diallel, without reciprocals, were assessed to study the genetic control of sweet pepper leaf and fruit resistance to bacterial spot. The evaluations were carried out in Campos dos Goytacazes (RJ) in an area of the UENF/ PESAGRO-RIO partnership.

Five *Capsicum annuum* L. genotypes, three susceptible (UENF 1420, UENF 1421 and UENF 1422) and two resistant (BGH 3071 and BGH 1772) to bacterial spot were used. The choice of these parents was based on their reaction to bacterial spot described in the literature (Santos, 1995) and on their divergent morphological-agronomic characteristics.

All possible crosses among the five parents were performed in a greenhouse (without reciprocals) to obtain the hybrids. Flower buds were emasculated and then pollinated. The fruits from the crosses were harvested separately when ripe and the seeds manually removed.

Hybrids and parents, in a total of 15 genotypes (treatments) were cultivated in a greenhouse, in 5 liter plastic pots in substrate treated with methyl bromide. A randomized complete block experimental design was used, with six and eight replications for RBS in leaves and in fruits, respectively. Each plot was represented by one plant per pot.

Isolate ENA 4135, which had been analyzed for virulence in preliminary experiments, was cultivated in liquid DYGS medium (Rodrigues Neto et al., 1986; Carmo et al., 1996) for about 39 hours. Inoculum was prepared using the following steps: a) the bacterial suspension was transferred to Petri dishes containing DYGS medium using a platinum blade; b) after a period of 36 to 48 hours growth at 28º±2ºC (Carmo et al., 1996), the bacterial colonies were suspended in sterile water and the cell concentration adjusted to 10^3 cells/ml for symptom quantification in a spectrophotometer using absorption of 600 nm (adapted from Bongiolo Neto et al., 1986).

Inoculation was performed on the third true leaf when the plants were approximately 40 days old. Infiltration of 0.5ml of bacterial suspension per leaf using the mesophyle method was used for leaf inoculation (Bongiolo Neto et al., 1986; Santos, 1995). Fruit inoculation was carried out using hypodermic needles previously placed in contact with bacteria cells.

Leaf assessments were carried out three weeks after inoculation by counting the number of pustules (x) in a 1.0 cm² area, according to the scale: score 1 = $0 \leq x \leq 5$; score 2 = $6 \leq x \leq 15$; score 3 = $16 \leq x \leq 30$; score 4 = $31 \leq x \leq 40$; score 5 = $41 \leq x \leq 50$ and score 6 = $x \geq 50$. Score one and six corresponded to resistance and susceptibility, respectively. Fruit assessment was performed seven days after inoculation. Lesions were measured at their greatest length (Y) using a digital pachymeter and the following scale was used adapted from studies with the common bean - *Xanthomonas axonopodis pv. phaseoli* pathosystem (Arnaud-Santana et al., 1994; Rodrigues, 1997): resistant = $0 \leq y \leq 1$ mm; moderately resistant = $1 \leq y \leq 2$ mm; moderately susceptible = $2 \leq y \leq 3$ mm; susceptible = $3 \leq y \leq 4$ mm; highly susceptible = $y \geq 4$ mm.

RESULTS AND DISCUSSION

Significant treatment mean squares were detected for all traits by the F test ($\alpha < 0.01$), indicating the presence of genetic variability among the genotypes. The experimental accuracy measured by the coefficient of variation was 12.79% and 18.05% for RBS on leaves and fruits, respectively.

Genotypes with RBS leaf scores above 3.0 were discarded because they did not show a satisfactory resistance level. The following genotypes were selected based on their small leaf scores: BGH 1772, BGH 3071, UENF 1420 x BGH 1772, UENF 1421 x BGH 3071.

Fruit RBS of 2.5, corresponding to the mid-point of

the adopted scale, was used as selection criteria. The following genotypes were selected: UENF 1421, BGH 3071, UENF 1420 x BGH 3071, UENF 1421 x BGH 3071 e UENF 1422 x BGH 3071.

Most of the hybrids obtained from BGH 3071 were selected based on their fruit RBS scores. However, UENF 1420 and BGH 1772 genotypes, which were selected based on leaf RBS scores, were not selected based on fruit score. On the other hand, UENF 1421, UENF 1420 x BGH 3071, UENF 1422 x BGH 3071 and BGH 1772 x BGH 3071, which were not selected based on their leaf scores, were selected based on fruit RBS scores. The genotypes UENF 1420, UENF 1422, UENF 1420 x UENF 1421, UENF 1420 x UENF 1422, UENF 1421 x UENF 1422, UENF 1421 x BGH 1772 and UENF 1422 x BGH 1772 were not selected in any of the assessments.

Duncan's test applied on the leaf RBS mean scores distributed the genotypes in six groups, with the BGH 3071 parent and the UENF 1421 x UENF 1422 hybrid representing the most resistant and most susceptible ones, respectively. The remaining genotypes showed intermediate resistance / susceptibility levels.

The Duncan test applied on the fruit RBS mean scores also distributed the genotypes in six groups, with BGH 3071 and UENF 1420 x UENF 1422 representing the most resistant and the most susceptible ones, respectively. The other genotypes showed intermediate resistance/susceptibility levels.

The presence of significant treatment differences allowed the genetic analyses of the data using the methodology proposed by Griffing (1956). Sum of the squares for treatments was partitioned in General Combining Ability (CGA) and Specific Combining Ability (SCA) sum of squares, according to the scheme shown in Table I following the Method 2, Model 1 analysis proposed by Griffing (1956).

Table 1 shows that only the general combining ability effects were significant for both leaf and fruit resistance. This indicates that additive genetic effects are more important in the control of resistance to bacterial spot than non-additive effects.

Small magnitude of **gi** values, either positive or negative, indicated parents whose combination did not differ from the mean of all crosses in the diallel. However, parents with high **gi** values, positive or negative, were greatly superior or greatly inferior compared to the others in the diallel.

The GCA scores are indicators of the importance of the genes showing predominantly additive effect. Parents displaying high GCA scores should be potentially superior and may, therefore, be included in breeding programs for selection of new pure lines in advanced generations (Ramalho et al., 1993). However, in the case of leaf and fruit RBS, the situation is inverse due to the decreasing score scale used and, therefore, lower **gi** means indicate greater resistance to bacterial spot.

Table 1. Estimates of the mean squares among the *Capsicum annuum* L. genotypes (parents and their F1 hybrids of the diallel), the general and specific combining abilities (GCA and SCA) and the error variance and the estimates of the square values of the mean combining ability effects and error variance for reaction to bacterial spot (BS) in leaves and fruits, according to method 2, model 1 of Griffing (1956).

SV	RBS leaves		RBS fruits	
	DF	MS	DF	MS
Genotypes	14	0.2255	14	0.3699
GCA	4	0.5756[1/]	4	1.1328[1/]
SCA	10	0.0855	10	0.0647
Error	70	0.0625	98	0.1156
Mean Square of the Effects				
GCA		0.0122		0.0182
SCA		0.0038		0.0000
Error		0.0625		0.1156

[1/] Significant by the F-test, at 1% probability level.

According to the leaf RBS scores, only the BGH 1772 and BGH 3071 parents contributed to increase BS resistance. The BGH 3071 contribution was greater than that of BGH 1772. The other parents showed positive scores and, therefore, may not have contributed to resistance under the conditions of the experiment (Table 2).

Regarding the fruit RBS scores, only the UENF 1421 and BGH 3071 genotypes showed negative **gi** values, indicating that their combination contributed genetically towards greater resistance to BS. The contribution of BGH 3071 was greater than that of UENF 1421. The other parents did not contribute to resistance as they showed positive **gi** values (Table 2).

CONCLUSIONS

Noting that the results obtained were inherent to the test conditions and cannot be extrapolated to other

Table 2. Estimates of the general combining ability ($\hat{g}i$) for reaction to bacterial spot (BS) in leaves and fruits assessed in five *Capsicum annuum* L. genotypes and the standard deviations (SD) of the effects of two different parents.

Genotypes	RBS	
	Leaves	Fruits
1. UENF 1420	0.0326	0.1308
2. UENF 1421	0.0262	-0.0623
3. UENF 1422	0.1436	0.0852
4. BGH 1772	-0.0238	0.0661
5. BGH 3071	-0.1786	-0.2198
SD ($G_i - G_j$)	0.0545	0.0643

environments and populations, it was concluded that:

• BGH 1772 and BGH 3071 were the parents that most contributed to the increase in leaf resistance and BGH 1772 and UENF 1421 contributed most to the increase in fruit resistance for the population under study.

• The analysis of Griffing (1956) showed that only additive effects are involved in the control of the leaf and fruit resistance to BS.

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RESUMO

Análise Genética da Resistência à Mancha Bacteriana em Pimentão

A mancha bacteriana (MB) é considerada uma das mais importantes da cultura do pimentão, sendo causada pela bactéria *Xanthomonas axonopodis* pv. v*esicatoria*. Esta doença pode acarretar em grandes perdas na produção. Os métodos de controle químico e físico não são eficientes, e entre as medidas de controle recomendadas, destaca-se a resistência genética. Estudos genéticos da resistência são básicos para a definição dos métodos de melhoramento a serem adotados para cada caso. Neste trabalho avaliou-se a capacidade de combinação de cinco genótipos de pimentão quanto à resistência à MB, em folhas e frutos. A análise foi feita utilizando-se o esquema de cruzamentos dialélicos segundo o método II, modelo I, de Griffing (1956). A inoculação em folhas utilizou o método de infiltração. A inoculação nos frutos foi feita perfurando-se o fruto com uma agulha hipodérmica. A reação à MB foi avaliada (após três semanas da inoculação) por meio de uma escala de notas de 1 (resistente) a 6 (suscetível) para folhas. Para frutos, a avaliação foi realizada sete dias após a inoculação, segundo a escala de notas de 1 (resistente) a 5 (suscetível). A Capacidade Geral de Combinação (CGC) foi significativa tanto em folhas quanto em frutos. A capacidade específica de combinação não foi significativa para resistência à MB tanto em folhas quanto em frutos. De acordo com a análise de Griffing (1956), os parentais BGH1772 e BGH 3071 foram os que contribuíram para o incremento da resistência em folhas, e, BGH 1772 e UENF 1421 para a resistência em frutos.

REFERENCES

Allard, R. W. 1971. Princípios do melhoramento genético das plantas. Edgard Blucher, São Paulo.

Arnaud-Santana, E.; Coyne, D. P.; Eskridge, K. M. and Vidaver, A. K. 1994. Inheritance, low correlations of leaf, pod, and seed reactions to common blight disease in common beans, and implications for selection. J. Amer. Soc. Hort. Sci. 119:116-121.

Blank, A. F. 1997. Teste precoce da capacidade combinatória de linhagens de pimentão (*Capsicum annuum L.*.). Ph.D. Diss. Universidade Federal de Lavras, Lavras.

Boiteaux, L. S. and Lopes, C. A. 1993. Resistência de campo à podridão mole (*Erwinia* spp.) dos frutos de pimentão com a presença do gene *up*. Horticultura Brasileira. 11:64.

Bongiolo Neto, A.; Reifschneider, F. J. B. and Takatsu, A. 1986. Padronização de metodologia para avaliação da resistência em *Capsicum* spp. à *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye e da virulência de isolados da bactéria. Fitopatologia Brasileira. 12:190-193.

Carmo, M. G. F. do; Kimura, O.; Maffia, L. A. and Carvalho, A. de O. de. 1996. Progresso da pústula bacteriana do pimentão, causada por *anthomonas*

campestris pv. *vesicatoria*, em condições de viveiro. Fitopatologia Brasileira. 21:62-70.

Cruz, C. D. and Regazzi, A. J. 1994. Modelos Biométricos aplicados ao melhoramento genético. Imprensa Universitária, Viçosa.

Griffing, B. 1956. A generalised treatment of the use of diallel crosses in quantitative inheritance. Heredity. 10:31-50.

Heinz, G. P.; Boiteaux, L. S.; Lima, M. F. and Pessoa, H. B. S. V. 1993. Resistência de frutos de *Capsicum chinense* a *Colletotrichum gloeosporioides*. Horticultura Brasileira. 28:236.

Kimura, O. 1984a. Melhoramento de pimentão visando à resistência à pústula bacteriana. Informe Agropecuário. 10:41-44.

Kimura, O. 1984b. Enfermidades bacterianas do pimentão. Informe Agropecuário. 10:113.

Kousik, C. S. and Ritchie, D. F. 1996. Disease potential of pepper bacterial spot pathogen races that overcome the *Bs2* gene for resistance. Phytopatholog*y*. 86:1336-1343.

Lopes, C. A. and Quezado-Soares, A. M. 1997. Doenças bacterianas das hortaliças. EMBRAPA, Brasília.

Nagai, H. 1983. Melhoramento do pimentão (*Capsicum annuum* L.) visando resistência ao vírus Y. Horticultura Brasileira. 1:3-9.

Ramalho, M. A. P.; Santos, J. B. dos and Zimmermann, M. J. de O. 1993. Genética quantitativa em plantas autógamas; aplicações ao melhoramento do feijoeiro. Editora da UFG, Goiânia.

Reifschneider, F. J. B. and Lopes, C. A. 1997. Resistência de plantas a fitobactérias. p.41-46. In: Palestras do Congresso Brasileiro de Fitopatologia, 30th, Poços de Caldas, 1997. Sociedade Brasileira de Fitopatologia.

Rodrigues Neto, J.; Malavolta Jr., V. A. and Victor, O. 1986. Meio simples para o isolamento e cultivo de *Xanthomonas campestris* pv. *citri* tipo B. Summa Phytopathologica. 12:16.

Rodrigues, R. 1997. Análise genética da resistência ao crestamento bacteriano comum e outras características agronômicas em *Phaseolus vulgaris* L. Ph.D. Diss. Universidade Estadual do Norte Fluminense, Campos dos Goytacazes.

Rodrigues, R. and Leal, N. R. 1991. Avaliação de cultivares de pimentão em duas regiões de cultivo do Estado do Rio de Janeiro. Comunicado Técnico 206. PESAGRO-RIO, Rio de Janeiro.

Sahin, F. and Miller, S. A. 1996. Characterization of Ohio strains of *Xanthomonas campestris* pv. *vesicatoria*, causal agent of bacterial spot of pepper. Plant Disease. 80:773-778.

Sahin, F. and Miller, S. A. 1998. Resistance in *Capsicum pubescens* to *Xanthomonas campestris* pv. *vesicatoria* pepper race 6. Plant Disease. 82:794-799.

Salgado, C. L. and Tokeshi, H. 1980. Doenças das solanáceas (berinjela, jiló, pimentão e pimenta). p.497-510. In: Galli, F. (Coord.) Manual de Fitopatologia: doenças das plantas cultivadas. Ed. Agronômica Ceres, São Paulo.

Santos, A. S. 1995. Caracterização morfológica de germoplasma do gên*ero* Capsicum e detecção de fontes de resistência à *Colletotricluum gloeosporioides* (Penzig) Penzig et Saccardo e à *anthomonas campestris pv. vesicatoria* (Doidge) Dye. M.S. Thesis. Universidade Federal Rural do Rio de Janeiro, Seropédica.

Soares, L. 1995. Divergência genética com base em componentes principais modificados e análise dialélica em pimentão (*Capsicum annuum* L.). D.S. Thesis. Universidade Federal de Viçosa, Viçosa.

Stall, R. E. 1997. Breeding for resistance to bacterial diseases of plants. p.51-56. In: Palestras do Congresso Brasileiro de Fitopatologia, 30th, Poços de Caldas. 1997. Sociedade Brasileira de Fitopatologia.

Vauterin, L.; Hoste, B.; Kersters, K. and Swings, J. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45:472-489.

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