



## ***Xanthomonas axonopodis* pv. *phaseoli* resistance in common bean (*Phaseolus vulgaris* L.) recombinant inbred lines**

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**ABSTRACT** - Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli*, *Xap*, is considered one of the major bacterial diseases affecting common bean. This disease is widely distributed in most locations where common bean is cultivated. Chemical control of CBB is ineffective; the use of resistant varieties is therefore an adequate method of disease control. The objective of the present study was to evaluate CBB resistance in advanced ( $F_6$  and  $F_7$ ) generations, derived from the cross HAB-52 (susceptible) x BAC-6 (resistant) and inoculated with the *Xap* CNF-15 isolate, as well as to estimate the genetic parameters related to resistance. In the  $F_6$  e  $F_7$  generations, the narrow-sense heritabilities for disease incidence and variation index were, respectively, 80.0% and 1.17, and 88.3% and 1.64. These results demonstrate potential of these advanced populations for selecting common bean CBB resistant varieties.

**Key words:** disease resistance, genetic parameters, RIL's (Recombinant Inbred Lines), Common bacterial blight.

### **INTRODUCTION**

Common bean (*Phaseolus vulgaris* L.) is one of the most important crops from social, economical and nutritional viewpoints (Yu et al. 2000). Common bacterial blight, caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), has become one of the major bacterial diseases affecting this crop (Silva et al. 1999). Chemical control is ineffective; the use of resistant varieties is therefore an attractive alternative, especially for small farmers. Although the use of resistant varieties is considered the most simple and economical means of control, this practice is sometimes impaired by pathogen variability in combination with different reactions in leaves and pods, as well as complex inheritance (Arnaud-Santana et al. 1994).

The strategy of obtaining recombinant inbred lines (RIL's), although considered time-consuming, is an excellent approach to increase genetic gains in selection procedures. The additive variance among RIL's is twice as high compared to the original  $F_2$  population. The objective of the present work was to evaluate advanced populations ( $F_6$  and  $F_7$ ) by artificial inoculation with a bacterial suspension of *Xap* CNF-15. RIL's from the cross HAB-52 (susceptible snap bean) x BAC-6 (resistant common bean) were used. BAC-6 has been used in many common bean breeding programs aiming at CBB resistant varieties (Mohan et al. 1981, Aggour et al. 1989, Arnaud-Santana et al. 1994, Jung et al. 1996, Freyre et al. 1998).

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Leaf and pod resistance, as well as genetic parameters were evaluated, as follows: heritability on family-mean basis, which provided more security in the selection of resistant genotypes; coefficients of variation (genetic and experimental) and the variation index, which allows an evaluation of the variability within the analyzed population. As an advanced population, it can be studied with other isolates in different environments, thus leading to a more profound understanding of the behavior of individuals in the population and of the possible contribution of each to common bean breeding programs.

## MATERIALS AND METHODS

### Plant material

The plant material was derived from a cross between HAB-52 (susceptible snap bean) and BAC-6 (resistant common bean). This cross was first studied by Rodrigues et al. (1999), when investigating the combining ability of a group of genotypes and respective crosses for CBB resistance, as well as for other agronomic traits. The advancement of the generations (F<sub>1</sub>-F<sub>7</sub>) was conducted on the experimental area of the UENF-State University of Northern Rio de Janeiro at the Experimental Station of PESAGRO-RJ, in Campos, Rio de Janeiro, Brazil. The F<sub>2</sub> and F<sub>3</sub> generations had been conducted and studied by Bressan-Smith (1998) and Santos (2000), respectively, and the F<sub>4</sub>-F<sub>7</sub> generations were conducted in the present study. F<sub>3</sub>-F<sub>7</sub> generations were obtained by the SSD (Single Seed Descent) method (Brim 1966). However, it is worth mentioning that along the generations, some families were lost due to phytosanitary problems. This is an important observation, considering that it could be related to this cross in particular. All agricultural recommendations for common bean cultivation were taken into consideration (Sartorato et al. 1996).

### Inoculum preparation

The CNF-15 isolate, considered highly pathogenic (Rava 1984), was used for the inoculation of leaves and pods. It was replicated in Petri dishes containing DYGS solid culture media (dextrose, peptone, yeast extract, K<sub>2</sub>HPO<sub>4</sub>, MgSO<sub>4</sub> and glutamic acid) (Rodrigues Neto et al. 1986). The bacteria were cultivated in DYGS liquid media for approximately 30 hours under agitation. The bacterial suspension was then grown in solid DYGS media at 28 °C. After 36 hours, the bacterial suspension was prepared adding sterilized salt solution (0.85% sodium chloride), and adjusted to the concentration of 10<sup>7</sup> cfu mL<sup>-1</sup> (Valladares-Sanches et al. 1979) using the SPEKOL/ZEISS UVIS spectrophotometer (absorbance: 640 nm) (Arnaud-Santana et al. 1994). Inoculations were established on the same day the cell suspension had been prepared.

### Experimental design

The trials were conducted September through December 2000 (for population F<sub>6</sub>) and May through August 2001 (for population

F<sub>7</sub>), in a randomized complete block design, with three replications, three sets (each with approximately 37 families, in 109 families, including the parents), and three plants per plot. The trials were separated into sets in order to minimize errors at inoculations and evaluations (Hallauer and Miranda Filho 1981). In view of the large number of families, and that these procedures were time-consuming, the use of sets simplified the evaluation and offered a better quality of the final results.

### Leaf and pod inoculation

For leaf inoculation, scissors contaminated with the bacterial suspension (10<sup>7</sup> cfu mL<sup>-1</sup>) were used to cut the leaflets (Santos 2000). Two leaflets of each plant were inoculated (always the middle leaflet). Pod inoculations were carried out as soon as all families presented developing pods with seeds. Two perforations were made using contaminated hypodermic needles in the middle section of the pods (between two seeds in formation), inoculating two pods per plant (Aggour et al. 1989). Plants were inoculated 38 days after sowing.

### Evaluation of *Xap* reaction in leaves and pods

Evaluations of the leaves were carried out seven days after inoculation (F<sub>6</sub> summer - average temperature 24.7 °C) and 11 days after inoculation (F<sub>7</sub> winter - average temperature 22.3 °C), following a scale adapted by Pastor-Corrales et al. (1981) varying from 1 to 5: 1 = no symptoms; 2 = 1 to 5% necrosis; 3 = 6 to 25% necrosis; 4 = 26 to 50% necrosis and 5 = > 50% necrosis. The final score of evaluations carried out in two individuals was considered the disease index (DI), given by the average of the two evaluations, for both leaves of each plant.

Pod evaluations were carried out 11 (F<sub>6</sub> - summer) and 15 days after inoculation (F<sub>7</sub> - winter). The maximum diameter of each lesion was measured, whereas the average of the four lesions (2 perforations per pod/2 pods per plant) was considered the final score - diameter of the pod lesion (DPL).

### Estimates of genetic parameters

The estimated genetic parameters in populations F<sub>6</sub> and F<sub>7</sub> were:

genotypic variance ( $\hat{\sigma}_g^2$ )

$$\hat{\sigma}_g^2 = (MS_2 - MS_1)/r$$

where

$$MS_1 = \hat{\sigma}^2$$

$$MS_2 = \hat{\sigma}^2 + r\hat{\sigma}_g^2$$

phenotypic variance ( $\hat{\sigma}_p^2$ )

$$\hat{\sigma}_p^2 = \hat{\sigma}_g^2 + (\hat{\sigma}^2)/r$$

narrow-sense heritability on family-mean basis ( $\hat{h}_m^2$ ).

$$\hat{h}_m^2 = \hat{\sigma}_g^2 / \hat{\sigma}_p^2 \text{ (whereas } \hat{\sigma}_g^2 = 2\hat{\sigma}_a^2 \text{)}$$

genetic variation coefficient ( $VC_g$ )

$$VC_g = (\sqrt{\hat{\sigma}_g^2 / \bar{x}}).100$$

experimental variation coefficient ( $VC_e$ )

$$VC_e = (\hat{\sigma}^2 / \bar{x}).100$$

variation index (VI)

$$VI = VC_g / VC_e$$

## RESULTS AND DISCUSSION

### Analysis of variance for CBB resistance

The analysis of variance (Table 1) shows significant difference for DI as well as for DPL in both generations ( $F_6$  and  $F_7$ ), also demonstrating the existence of genetic variability among the evaluated genotypes. The variation coefficients seem to be quite adequate, considering that the trial was carried out under field conditions and the complexity of the disease.

### Estimates of genetic parameters

The narrow-sense heritability on family-mean basis ( $\hat{h}_m^2$ ) and the variation index (VI) were considerably high (80.0% and 1.17 in  $F_6$  and 88.3% and 1.64 in  $F_7$ , respectively, - Table 2),

compared to the  $F_3$  generation (Santos 2000), whereas the estimated values were 26.85% and 0.26 for  $\hat{h}_m^2$  and VI, respectively.

Many studies regarding heritability and CBB resistance reaction can be found in literature - all with certain differences among results. Some studies show low heritability in leaf and pod reactions (Arnaud-Santana et al. 1994, Ariyaranthne et al. 1998), whereas high heritabilities were reported by Pompeu and Crowder (1972). These results demonstrate that there is still a lot of research to be done regarding the complexity of this disease. Actually, the heritability values depend on different aspects such as the population in consideration, environmental conditions, the experimental design, precision of data collection and, most important, the genetic complexity of the trait under study. Therefore, differences in heritability results for the same trait are quite common. An important aspect is that the heritability values under the investigation conditions (population, environment, methodology, etc) must be well known to establish selection procedures aiming at genetic gains.

Heritability on family-mean basis demonstrated that the fact of the evaluations being carried out with advanced materials contributed to its increase, enabling a more accurate selection of superior genotypes. It was also noticed that not only different environments and different study populations, but also the inoculation method influence results (Rodrigues

**Table 1.** Analysis of variance for CBB resistance under field conditions in common bean recombinant inbred lines ( $F_6$  and  $F_7$  generations) inoculated with isolate CNF-15 *Xap*

Sources	Mean square							
	df	Leaves- $F_6$ (DI <sub>1</sub> )	df	Pods- $F_6$ (DPL <sub>1</sub> )	df	Leaves- $F_7$ (DI <sub>2</sub> )	df	Pods- $F_7$ (DPL <sub>2</sub> )
Set	2	0.57	2	0.23	2	0.65	2	1.30
Replications	6	2.53	6	0.93	6	6.03	6	0.38
Gen (Set)	97	1.26**	101	0.52**	99	1.68**	97	0.35**
Error	192	0.24	165	0.14	174	0.18	140	0.23
VC (%)		17.4		29.9		23.1		30.4
R <sup>2</sup>		75.3		70.3		67.9		54.5

\*\* Significant at 1% of probability; DI<sub>1</sub>: disease index for leaf inoculation in  $F_6$ ; DPL<sub>1</sub>: diameter of pod lesion in  $F_6$ ; DI<sub>2</sub>: disease index for leaf inoculation in  $F_7$ ; DPL<sub>2</sub>: diameter of pod lesion in  $F_7$ . R<sup>2</sup>: precision of the adjustment to the mathematical model.

**Table 2.** Estimates of genetic parameters evaluated for disease index (DI) and diameter of pod lesion (DPL) in common bean RILs inoculated with isolate CNF-15 *Xap*

Characteristics	$\hat{\sigma}^2$	$\hat{h}_m^2$	$VC_e$	$VC_g$	VI
		%			
Leaves $F_6$ (DI <sub>1</sub> )	0.24	80.0	17.4	20.5	1.17
Pods $F_6$ (DPL <sub>1</sub> )	0.14	71.0	29.9	27.6	0.92
Leaves $F_7$ (DI <sub>2</sub> )	0.18	88.3	23.1	38.4	1.64
Pods $F_7$ (DPL <sub>2</sub> )	0.24	34.0	30.4	12.5	0.41

$\hat{\sigma}^2$ : residual variance;  $\hat{h}_m^2$ : narrow-sense heritability on family-mean basis;  $VC_e$ : experimental variation coefficient;  $VC_g$ : genetic variation coefficient; and VI: variation index.

et al. 1999, Santos 2000). As to the VI values, when compared to the ones obtained for the F<sub>3</sub> population, a considerable increase is observed (VI ≥ 1.00 means that there is genetic variability within the population to be explored). This situation demonstrates the great potential of this population in genetic variability, that is, its existence and the possibility to discover promising genotypes by this means. The experimental variation coefficients for leaf inoculation (F<sub>6</sub> and F<sub>7</sub>) were lower than the genetic coefficient of variation, which had been expected. The same was not true, however, for pod lesion, probably due to angular leaf spot (*Phaeoisariopsis griseola* Sacc. Ferr.) symptoms. These masked the *Xanthomonas axonopodis* pv. *phaseoli* lesions, and therefore made it difficult to obtain precise results, a condition also reported by Santos (2000). In general terms, the family-mean heritability values were quite

high, especially because of the RIL's strategy. The additive variance was doubled, compared to the corresponding F<sub>2</sub> population.

However, these results clearly demonstrate that more research regarding the pathogenicity and more tests in different environments, along with the identification of desirable combinations as to the reaction to the disease will certainly contribute to a better understanding of the complexity of CBB in common beans.

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## Resistência à *Xanthomonas axonopodis* pv. *phaseoli* em linhagens recombinantes de feijoeiro (*Phaseolus vulgaris* L.)

**RESUMO** - *Dentre as doenças bacterianas que acometem o feijoeiro, o crestamento bacteriano comum (CBC), causado por Xanthomonas axonopodis* pv. *phaseoli*, Xap, merece destaque. A doença é amplamente distribuída na área de cultivo do feijoeiro. Seu controle químico é ineficiente, tornando o uso de variedades resistentes a medida mais adequada. No presente estudo avaliou-se a resistência ao CBC em gerações avançadas (F<sub>6</sub> e F<sub>7</sub>), provenientes do cruzamento HAB-52 (suscetível) x BAC-6 (resistente) inoculadas com o isolado de Xap CNF-15, bem como se estimaram os parâmetros genéticos relacionados à resistência. As estimativas de herdabilidade no sentido restrito para índice de doença e de índice de variação em F<sub>6</sub> e F<sub>7</sub> foram de 80,0% e 1,17 e de 88,3% e 1,64, respectivamente. Estas estimativas demonstram o potencial destas populações avançadas para a seleção de variedades de feijoeiro resistentes ao CBC.

**Palavras-chave:** resistência a doença, estimativas de parâmetros genéticos, RIL's - linhagens endogâmicas recombinantes, crestamento bacteriano comum.

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