Genetic Linkage Map of *Phaseolus vulgaris* and Identification of QTLs Responsible for Resistance to X*anthomonas axonopodis* pv. p*haseoli*[*]

Amaury S. Santos[1], Ricardo E. Bressan-Smith[2], Messias G. Pereira[2], Rosana Rodrigues[2] & Claudia F. Ferreira[3]

[1]Instituto Biológico, Cx Postal 70, Campinas, SP, CEP 13001-970, tel. (19) 3252 1657, e-mail: amaury@biologico.br; [2]Laboratório de Melhoramento Genético Vegetal, Universidade Estadual do Norte Fluminense, Av. Alberto Lamego, 2000, Campos dos Goytacazes, RJ, CEP 28015-620, e-mail: brsmith@uenf.br; [3]Embrapa Mandioca e Fruticultura, Cx. Postal 007, CEP44380-000, Cruz das Almas, BA

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Corresponding author: Amaury da Silva dos Santos

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ABSTRACT

Common bean (*Phaseolus vulgaris*) cultivars with a high degree of resistance to *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) are not available in Brazil. Despite many studies, a low degree of resistance to *Xap* continues to exist due to its complex genetic inheritance, which is not well known. The objectives of this research were to complement a common bean genetic map based on the cross between a susceptible genotype 'HAB-52' and a resistant genotype 'BAC-6', and to map and analyze genomic regions (quantitative trait loci – QTLs) related to *Xap* resistance. Eleven linkage groups were determined using 143 RAPD markers, covering 1,234.5 cM of the genome. This map was used to detect QTLs associated with *Xap* resistance on leaves and pods. The averages of disease severity on leaves (represented by the transformed disease index – TDI) and pods (represented by the diameter of lesion on pods – DLP) were added to the data of the linkage map. Five TDI QTLs and only one LDP QTL were detected. The TDI QTLs were placed in the A, B, G and J linkage groups, with phenotypic variations ranging from 12.7 to 71.6%. The DLP QTL explained 12.9% of the phenotypic variation and was mapped in a distinct linkage group. These results indicate that there are different genes involved in the control of resistance on leaves and pods.

Additional key words: common bean, quantitative trait loci, common bacterial blight, disease resistance.

RESUMO

Mapa genético de ligação de *Phaseolus vulgaris* e identificação de QTLs responsáveis pela resistência à *Xanthomonas axonopodis* pv. *phaseoli*

Não há disponibilidade de cultivares comerciais de feijoeiro (*Phaseolus vulgaris*) com alto nível de resistência à *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) no Brasil. Isto se deve à complexa herança de resistência que, apesar dos diversos estudos efetuados, ainda não foi completamente elucidada. Os objetivos deste trabalho foram complementar um mapa genético do feijoeiro, construído com base no cruzamento entre um genótipo susceptível 'HAB-52' e um genótipo resistente 'BAC-6' e mapear e analisar regiões genômicas (QTLs) relacionadas com a resistência à *Xap*. Onze grupos de ligação foram identificados, por meio de 143 marcadores RAPD, cobrindo 1234,5 cM do genoma. Este mapa foi usado para detectar QTLs associados à resistência em folhas e em vagens a *Xap*. As médias de severidade de doenças em folhas e em vagens, representadas pelo índice de doença transformado (IDT) e pelo diâmetro de lesão em vagens (DLV), respectivamente, foram adicionados aos dados do mapa de ligação. Detectaram-se cinco QTLs para IDT e um para DLV. Os QTLs para IDT localizaram-se nos grupos de ligação A, B, G e J, com variância fenotípica variando de 12,7 a 71,6%. O QTL identificado para DLV, com variância fenotípica de 12,9%, localizou-se num grupo de ligação distinto, indicando que a reação de resistência em folhas e vagens é controlada por diferentes genes.

INTRODUCTION

Brazil is the world's largest producer of common bean and snap bean (*Phaseolus vulgaris* L.). Bean cultivation is also socially important because it is mainly carried out by small farmers. The level of crop technology used is often low, because farming is at the subsistence level where use of fertilizers and pesticides is rare. Diseases are important factors that interfere with bean yield. Bean common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith 1897) Vauterin, Hoste, Kersters & Swings 1995 (Young *et al*., 1996) (*Xap*), is the main bacterial disease

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affecting both the common bean and the snap bean in Brazil. Based on the social and economic implications of bean cultivars, adoption of resistant cultivars would be the simplest, most promising, most economically effective and ecologically sustainable method of disease control, especially when combined with the use of healthy seeds and crop practices that prevent the establishment of pathogens in the field.

Breeding cultivars for *Xap* resistance has proved difficult because of the influence of several factors on the complex interaction between bean and this bacterial pathogen. Several authors have reported that common bean resistance to *Xap* is genetically complex (Pompeu & Crowder, 1972; Valladares-Sanches *et al.*, 1979). In the past, classic quantitative genetics was the recommended tool for dealing with complex disease resistance traits. However, classic quantitative genetics is unsuitable for dissecting polygenic resistance in discrete genetic loci or in defining the function of individual genes responsive to disease resistance (Young, 1996). An adequate methodology for studying complex and polygenic forms of resistance to disease is known as QTLs (Quantitative trait loci) mapping, which is based on the use of DNA markers (Young, 1996).

The present study was carried out to identify QTLs associated with resistance to *Xap* through the construction of a genetic linkage map obtained by DNA markers and through the phenotypic analysis of the resistance in plants in the ongoing bean breeding program at the North Fluminense State University, Campos dos Goytacazes, RJ.

MATERIALS AND METHODS

The genotypes used in the present study are derived from the cross between 'HAB-52' (susceptible to *Xap*) and 'BAC-6' (resistant to *Xap*) carried out by Rodrigues (1997). The F1 generation was obtained and the plants selfed to produce the F2 generation, which were then selfed to produce the F3 families. A total of 88 F2 plants were used to construct the genetic linkage map and their F3 families were used to obtain the phenotypic data (resistance to *Xap*).

The genomic DNA was isolated according to the method described by Skroch & Neinhuis (1995) with modifications. The DNA was purified with RNAse (10 mg/ml) and its concentration assessed in a SPEKOL/SEISS UV-VIS spectrophotometer (Zeiss, Germany) at 260 nm. The amplifications were made with previously selected polymorphic primers. The amplification procedure used was one minute at 95 °C for initial denaturation, followed by 40 cycles, each cycle consisting of denaturation stages (1 min at 94 °C), primer annealing (1 min at 36 °C) and primer extension (2 min at 72 °C). After 40 cycles, there was a 7 min cycle at 72 °C for final extension. The electrophoresis conditions were 1.6% agarose gel in a horizontal electrophoresis cube submitted to 110 V for 3 h. After electrophoresis, the gel was bathed in ethidium bromide (0.5 µl/ml) and photodocumented in the Eagle Eye II system (Stratagene, USA) under ultraviolet light. The primers that showed at least three polymorphic bands were used for the linkage map construction.

The linkage analysis was carried out by the MAPMAKER/EXP software (Lander *et al.*, 1987) using 143 RAPD loci and four morphological markers (position of the tip of the flower, flower color, leaf surface and growth habit) recorded from 88 plants of the F2 population. The genotypes in the loci containing dominant alleles were standardized as follows: a) when the band came from 'HAB-52' (there was no band in 'BAC-6') the compositions were D and B for presence or absence, respectively; b) when the band came from 'BAC-6' (there was no band in 'HAB-52') the compositions were C and A for presence and absence, respectively. The segregation of each marker was tested for possible deviations from the 3:1 ratio between loci DB and CA (RAPD is dominant) by the chi-square test (χ^2).

After an artificial inoculation in the field with the bacterium in the leaves (the leaf was cut with scissors) and pods (perforated with hypodermic needle), the 88 F3 families were scored for disease index (DI) on a scale from 1.0 to 5.0 and for maximum diameter of lesion on pods (DLP). The DI was transformed mathematically into arc-sin ($\sqrt{x/100}$) equation to obtain the transformed disease index (TDI). The TDI and DLP means were then added to the linkage map for QTL detection.

The interval mapping (Lander & Botstein, 1989) was performed by the MAPMAKER/QTL 1.1 software (Paterson *et al.*, 1988) calculating and compiling the LOD scores at each 2 cM of the map. For each QTL identified, the MAPMAKER/QTL calculated the interval of confidence around the peak indicating the regions where the probability of significance is ten-fold smaller than the most probable position (Paterson *et al.*, 1991b).

RESULTS AND DISCUSSION

One hundred and four of the 143 polymorphic RAPD markers detected were mapped in 11 linkage groups (Figure 1). The linkage map reported in the present study spanned 1,234.5 cM with a mean interval of 11.87 cM (Table 1). Other available maps are those of Jung *et al.* (1996) with 545 cM distributed in eight groups with a mean interval of 6.9 cM; Nodari *et al.* (1993) with 827 cM and a mean interval of 6.5 cM; Vallejos *et al.* (1992) with 963 cM and a mean interval of 3.95 cM; Freyre *et al.* (1998) with 1,226 cM and a mean interval of 2.17 cM; and Ariyarathne *et al.* (1999) with 755 cM distributed in 11 linkage groups with a mean interval of 4.33 cM.

The expected number of linkage groups is 11, which corresponds to the haploid number of chromosomes of *P. vulgaris*. This agrees with the result obtained in the present study. Differences in the number of linkage groups are reported in practically all the linkage maps published on this species. Freyre *et al.* (1998) worked with a map in common bean spanning 1,226 cM using morphological, isoenzymatic, RFLP and RAPD markers. The size of the map reached by these

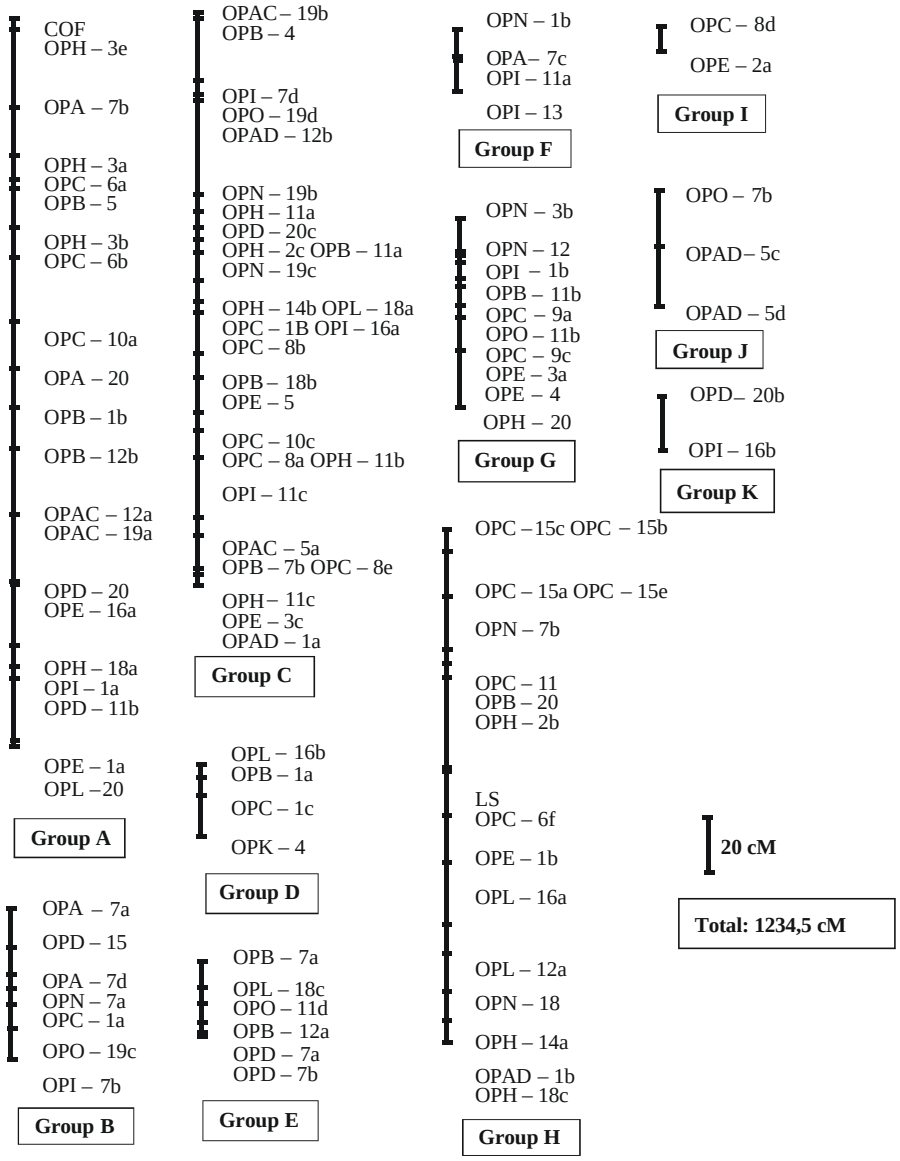

FIG. 1 - *Phaseolus vulgaris* partial linkage map. Using MAPMAKER 3.0 'software'. One hundred and two RAPD markers and two morphological markers (COF-color of flower and LS-leaf surface) were associated with 11 linkage groups (A-K). The letters that follow the designations of the RAPD markers, mean the order at which the polymorphic markers appear on the gel, being classified from the largest to the smallest amplified fragment.

authors is very close to the estimated value of 1,200 cM by Vallejos *et al.* (1992). On the other hand, Boscariol *et al.* (1998) used three types of markers (RFLP, RAPD and SCAR) that covered only 820.8 cM. The discrepancies observed in common bean maps constructed by using different types of markers (RFLP, RAPD, SCAR, AFLP, isoenzymes, morphological, etc.) indicate that the genome is still not completely saturated with marker loci (Boscariol *et al.*, 1998).

A high proportion of segregation distortion was obtained in this study (Table 1). Indeed, many studies have shown this tendency (Jung *et al.*, 1996; Jung *et al.*, 1997; Freyre *et al.*, 1998; Ariyarathne *et al.*, 1999). However, Nodari *et al.* (1993) and Vallejos *et al.* (1992) reported low segregation distortion by using RFLP based maps. As RAPD based maps usually generate high segregation distortion, this could have resulted from non-random distribution in the genetic map, occurrence of genetic drift, natural selection, presence of incompatibility in a specific genomic region, occurrence of problems in the gametogenesis, or seed development and plant growth (Jung *et al.*, 1997).

According to the localization of the QTLs and the description of their effects affecting the resistance trait to *Xap* (Table 2), the QTLs for the two resistance traits assessed (transformed disease index and pod lesion diameter) were

TABLE 1 - Description of the *Phaseolus vulgaris* linkage map consisting of 102 RAPD markers and two morphological markers segregating in the F_2 population from the cross between 'HAB-52' and 'BAC-6'

Linkage group	Number of markers	Genetic distance [a]	Average distance [b]	Segregation distortion [c]
A	21	357.7	17.03	1
B	7	76.9	10.98	1
C	28	277.5	9.91	10
D	4	33.7	8.42	2
E	6	32.3	5.38	0
F	4	35.6	8.91	0
G	10	87.5	8.75	4
H	17	238.6	14.03	0
I	2	9.6	4.80	0
J	3	56.7	18.90	0
K	2	28.4	14.20	1
Mapped markers	104	1234.5	11.87	19
Unmapped markers	39			13
Total	143			32

[a] Distance on the map in cM
[b] Average distance (cM) between adjacent markers
[c] Number of markers deviating from the expected segregation ratio 3:1 ($p < 0.05$) -mapped markers (22.37%) and unmapped markers (18.27%).

identified: five for TDI and one for DLP. Of the five QTLs detected for TDI, three were located in a different linkage group. The two QTLs found in the J linkage group are at a shorter distance than 50 cM and are, therefore, probably linked.

The QTL detected in DLP is found in a different linkage group from those found in TDI. This result indicates the absence of relationship between the resistance reactions to *Xap* in leaves and pods, which is in agreement with the Pearson correlation analyses where a very low correlation of DLP with TDI was observed (0.100). The existence of *Xap* resistant genes specific for determined plant organs controlling resistance in leaves and pods has been suggested by several authors based on different reactions to *Xap* isolates. Jung *et al.* (1997) studied two *Xap* isolates and observed that there was a smaller correlation among the reactions to *Xap* in leaves, pods and seeds for one isolate than for the other isolate. This suggests the presence of significant specific interaction effects between isolate and plant organ. Ariyarathne *et al.* (1999), however, reported the existence of a common gene or a gene cluster controlling the resistance reaction in leaves and pods. Hypotheses suggesting few genes controlling resistance to *Xap* infection on common bean leaves and pods have been presented in several independent studies (Valladares-Sanches *et al.*, 1979; Arnaud-Santana *et al.*, 1994).

The comparison of the direction of the QTLs responses (Table 2) shows that both parents contribute with favorable alleles to increase the resistance of their progenies. It was found for TDI that out of five declared QTLs, three received favorable alleles from 'BAC-6' and others from 'HAB-52'. The contribution of the susceptible parent for higher resistance was also detected by Boscariol *et al.* (1998). These are called cryptic genetic effects that is, phenotypic effects of alleles stemming from the susceptible parent. The cryptic effects that run contrary to what is expected are commonly reported in the literature (Young, 1996).

The genetic action of partial dominance for DLP is in line with the result obtained by Pompeu & Crowder (1972), who determined that *Xap* resistance is expressed by few genes

TABLE 2 - Location, effect and action of QTLs affecting common bean*Xanthomonas axonopodis* pv. *phaseoli* resistance traits in bean (*Phaseolus vulgaris*) (transformed disease index – TDI and diameter of lesion on pods - DLP)

Trait	Linkage group	R^2 (%)	LOD[a]	Gene effect[b]		d/a	Gene action
				a	d		
Transformed disease index (TDI)	A	12.7	2.22	0.9710	0.8159	0.84	DP
	B	67.2	2.41	-1.068	-2.724	2.55	SD
	G	51.7	2.75	1.059	-2.356	-2.22	SD
	J	71.6	2.87	-0.393	3.237	-8.24	SD
	J	68.7	2.38	-0.454	3.167	-6.97	SD
Diameter of lesion on pods (DLP)	D	12.9	2.14	0.7454	0.6974	0.93	DP

[a] Minimum LOD value
[b] Additive effects are associated with the 'BAC-6' allele. The negative sign indicates that the allele from 'BAC-6' decreases the mean value of the trait, therefore, increases resistance.

showing a partial dominance mean effect. It is possible that the detected QTL for DLP expresses a large main effect while others, showing smaller effects may have not been detected in this analysis. Eskridge & Coyne (1996) estimated at 5.2 and 1.1 the number of genes involved in leaf and pod resistance, respectively, in progenies derived from the XAN-159 x PC-50 cross. Jung *et al*. (1997) worked with recombinant lines derived from 'XAN-159' and 'PC-50' and detected one QTL strongly associated with resistance to *Xap* in leaves and others with smaller effects, thus confirming the hypothesis of Eskridge & Coyne (1996).

The use of new types of markers (AFLP, for example) and the trial of the genotypes in advanced generations may optimize a larger coverage by the map, facilitate the detection of new QTLs associated with resistance to *Xap* and also with other agronomically important quantitative traits. Many studies have indicated variation in the quantitative traits due to their sensitivity to environmental effects and to the genetic background of the genotypes used. Studies on tomatoes (*Lycopersicon esculentum* Mill) indicate that not all QTLs are detected in all the environments, but a few QTLs are always expressed regardless of the environmental conditions (Paterson *et al.*, 1991a). Thus, the assessment of QTLs in several populations and environments should be essential to confirm the use of QTLs in future genetic breeding programs. Until now, two studies in the literature have reported the confirmation of QTLs in different environments and populations (Jung *et al.*, 1999; Park *et al.*, 1999).

All markers associated with resistance reaction in leaves and pods detected in the present research should be studied in advanced generations, using several loci and different *Xap* isolates and, in the future, tested on other populations with different genetic backgrounds prior to the use in selection by DNA markers. This will ensure efficiency in common bean genetic breeding programs for resistance to *X. axonopodis* pv. *phaseoli.*

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