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QTL mapping for angular leaf spot in common bean using microsatellite markers

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ABSTRACT - *QTL identification is the first step in the application of molecular-marker- assisted selection in breeding. Common bean molecular maps are already available and QTL and, recently, SSR markers have been identified. The objective of this study was to identify QTL for reaction to angular leaf spot using segregating families from cross Jalo EEP 558 x Small White using SSR. These families presented significant differences for the trait. High heritability estimates were obtained. Environment effects and the interaction genotypes x environments influenced the trait expression. A map with 400.1 cM was established with 24 markers arranged in eight linkage groups with a mean length of 50.01 cM, and mean distance between adjacent markers of 25.01 cM. It was possible to identify steady QTL and associate them to a high percentage of phenotypic variance for reaction to angular leaf spot. BM210 and BM146 were the most outstanding markers.*

Key words: *Phaseolus vulgaris*, *Phaeoisariopsis griseola*, SSR

INTRODUCTION

Linkage maps allow us to deepen the understanding of the genome of a species and the identification of genes or genomic regions, denominated QTL, which control traits of economical importance. The phenotypic expression of these genes can have a discrete or continuous distribution; in the latter case the trait is generally controlled by more than one gene.

Among the markers applied for mapping those based on DNA polymorphism, especially the SSR (Simple Sequence Repeat) are of particular importance since they combine low cost with high repeatability of results and high polymorphism. SSR primers of common bean have recently become available, making the application of this tool in QTL mapping and identification possible. However to date few SSR loci have been inserted into the integrated map of common bean.

QTL identification is highly applicable in plant improvement since indirect selection based on molecular markers, uninfluenced by the environment in their genotypic manifestation, is advantageous if there is a strong linkage between QTL and the marker. In this case, one can use markers associated to favorable alleles of traits

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of problematic measurement for selection. The cost of molecular evaluation must also be considered, the percentage of phenotypic variation explained by the markers as well as the interaction QTL x environments when deciding on whether to adopt indirect selection based on molecular markers.

Angular leaf spot was once considered a disease of little importance for common bean; however, with the year-round cultivation the inoculum has increased in the field and more favorable environmental conditions for the development of the disease have increased its importance. Resistance to angular leaf spot is of the vertical (Ferreira et al. 1999) and horizontal type. Studies on the genetic control of reaction to angular leaf spot are not yet conclusive (Bett and Michaels 1995, Rezende et al. 1999, Faleiro et al. 2003). Melo et al. (2002) observed heritability for reaction to angular leaf spot varying from 44.44 to 58.86% and suggested that in the case of low heritability marker-assisted selection is more advantageous; they verified the presence of interaction genotypes x environments of the complex type; through the process of linear regression they found ten QTL associated to the reaction to angular leaf spot and two QTL by the method of composite interval mapping. They therefore suggested the use of primers OPN-02 and OPN-07 in assisted selection for reaction to angular leaf spot. RAPD markers were also used by Faleiro et al. (2003) who identified, in one and the same linkage group, two QTL for the reaction to angular leaf spot. SSR markers and RAPD were recently associated to angular leaf spot using line ESAL 550 (Silva et al. 2003).

The objective of this study was the identification of QTL related with the reaction to angular leaf spot using SSR markers and families derived from the crossing of the varieties Jalo EEP 559 and Small White.

MATERIAL AND METHODS

The parents used in this study were cultivars Jalo EEP 558 and Small White which are highly divergent in their reaction to angular leaf spot. Cultivar Jalo reacts completely tolerant, while cultivar Small White is highly susceptible to the pathogen. The cultivars were hybridized according to the methodology proposed by Ramalho et al. (1993) at the Universidade Federal de Lavras (UFLA) and once the F_2 generation was obtained, 142 plants were separated. From each F_2 plant, DNA was extracted for the molecular evaluation and one family obtained for phenotypic evaluations.

Phenotypic evaluations were realized on the field using families $F_{2:4}$ and $F_{2:6.}$ The trials were conducted at UFLA, Lavras-MG and on the experimental farm of the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG) in Lambari-MG. Four trials were set up, two each during the dry period of 2002 and 2003 in Lavras and in Lambari. The experimental design was a 12 x 12 simple lattice, in which the treatments consisted of the parents and 142 families derived from crossing Jalo EEP 558 x Small White. Each experimental plot contained two 2 meter long rows spaced 0.5 m apart with 15 seeds meter^{-1}. Crop management, fertilization and irrigation were those regionally and conventionally used, without disease control. The reaction to angular leaf spot (*Phaeoisariopsis griseola*) at the plot level was evaluated according to a descriptive scale of grades varying from 1 to 5 (Table 1) using a methodology proposed by Rezende et al. (1999). The pathogen reaction was assessed in an independent evaluation through at least two evaluators, whose mean was considered for analysis.

Table 1. Descriptive scale of grades used to evaluate the reaction to angular leaf spot (adapted from Rezende et al. 1999)

Grade	Infected leaf area (%)
1	less than 1
2	1 to 5
3	5 to 20
4	20 to 60
5	60 to 100

Analyses of variance were realized considering each environment separately, followed by the joint analysis. Based on the mean squares obtained in the analyses of variance the phenotypic and genetic variances and broad-sense heritability were estimated. The interaction genotypes x environments was decomposed in simple and complex portions (Vencovsky and Barriga 1992).

The DNA of the F_2 plants and both parents was extracted according to the methodology of Nienhuis et al. (1995), with modifications. Genotyping was realized with the SSR primers published by Yu et al. (2000) and by Gaitán-Solís et al. (2002). Each reaction contained: 40 ng genomic DNA; 100 mM of each one of the desoxyribonucleotides (dATP, dCTP, dGTP, dTTP); 1 unit of the enzyme Taq DNA polymerase, 50 mM Tris, pH 8.3; 20 mM KCl; 2 mM $MgCl_{2;}$ 10 mg BSA; 0.25% Ficol 400; 10 mM tartrazine; and water to attain 20 mL. The amplification was realized in a Mastercycler Eppendorf thermocycler programmed for: ten minutes at 95 ^{0}C; 9 cycles at 94 ^{0}C for 20 seconds; 20 seconds for primer annealing at temperatures from 46 to 68 ^{0}C, according to the primer; 20 seconds at 72 ^{0}C; 25 cycles of 20 seconds at 94 ^{0}C; 20 seconds for primer

annealing at temperatures from 46 to 60 ^{0}C, according to the primer; 20 seconds at 72 ^{0}C; and 10 minutes at 72 ^{0}C. The DNA fragments were separated in 2.5% agarose gel or 3.5% special high resolution agarose depending on the difference of sizes of the amplified DNA fragments in the parents. Ethidium bromide was used for staining of the DNA fragments that were visualized under ultraviolet light.

SSR marker data were subjected to the chi-square test (P=0.05) to verify the adjustment to the expected segregation in the F_2 generation of 1:2:1, considering the codominant nature of the SSR markers. The criterion of Bonferroni was used to control the level of global significance (Bearzoti 2000). Markers that did not present distortion in the Mendelian segregation were used for the calculation of the recombination frequency and later of the genetic distance (in cM) by the Kosambi function. The program used for mapping was Mapmaker version 3.0 (Lander et al. 1987). A minimum LOD of 3.90 was adopted and a maximum of 0.50 of recombination frequency to determine the existence of linkage between markers.

Cultivar Jalo was used as standard in a two-stage QTL identification. In the first phase, all segregating markers, whether linked or not, were considered. In this phase, linkages between markers and QTL were evaluated by means of multiple stepwise regression. Markers associated to the traits under study and a probability of $F < 0.05$ were considered. At the second stage of the QTL identification, only constant markers of the linkage map were considered for composite interval mapping by program QTL Cartographer for Windows version 1.01 (Basten et al. 1999). In this analysis, the maximum LOD was determined by means of 1000 data permutations and a probability of 5% of random QTL identification was considered. In both phases, QTL identification was first based on the trait means in each individual environment and then on the means used in the joint analyses.

RESULTS AND DISCUSSION

In all evaluations cultivar Jalo EEP 558 presented excellent performance in relation to the reaction to angular leaf spot, with mean grades ranging from 1.02 to 1.74, representing less than 5% of the leaf area affected by the pathogen. Cultivar Small White presented grade means between 3.98 and 4.62, corresponding to the lesions caused by the pathogen on more than 20% of the leaf area. In the experiment mean, the treatments scored 2.92, very similar to the parents' grade mean of 2.90, which suggests, together with the distribution of frequency (Figure 1), polygenic inheritance. One must however take into consideration that Jalo EEP 558 has vertical resistance, owing to one dominant allele (Silva et al. 2003). Moreover, an intermediate reaction was observed in the evaluation of segregating families, even for monogenic resistance, because one grade mean had been attributed to the entire plot. These results may be due to the fact that some plants presented complete resistance and others high susceptibility; in this case the grade of the family was about the mean. Besides the monogenic control, digenic inheritance was suggested for the reaction to angular leaf spot (Bett and Michaels 1995, Rezende et al. 1999).

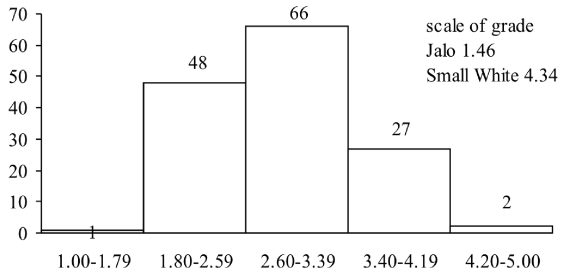

Figure 1. Distribution of frequency of the grades of angular leaf spot reaction considering experimental means

The coefficients of variation varied from 12.68 to 21.22%, indicating a smiilar experimental precision to the one observed in field experiments (Rezende et al. 1999, Melo et al. 2002). The treatment effect was significant for reaction to angular leaf spot in all experiments. The estimates of heritability were high varying from 57.83 to 79.52%, indicating some environmental influence on the phenotypic variation.

In the joint analysis, where the coefficient of variation was 17.56%, it was possible to verify the significant effect of the environment on the reaction to angular leaf spot, of its partitions and of the interaction treatments x locals and x years. It is worth mentioning the large contribution of the year to the environment effect; its square mean was four times higher than the one obtained for the local. This allows the supposition that the performance is most influenced by the year and not the local. The pathogenicity of angular leaf spot races found in Lavras and Lambari would therefore not be the strongest cause of the interaction. When considering the interaction treatments x environments and its partitionings, it is possible to verify influence on the reaction to angular leaf spot, similar contributions of the interaction treatments x locals and of treatments x years, and the absence of triple interaction. The partition of the interaction treatments x environments demonstrated once more the predominance of the complex part of the interaction, in this case 99.97%, which shows that the interaction treatments x environments was nearly

completely due to its complex portion, in line with the results obtained by Melo et al. (2002). Phenotypic correlations were however high, varying from 0.49 to 0.64, as well as the Spearman correlation which varied from 0.46 to 0.63. In the joint analysis, the heritability, free of the interaction genotypes x environments, was high, reaching 50.89%.

One hundred and five primer pairs of microsatellites published by Yu et al. (2000) and Gaitán-Sólis et al. (2002) were tested. Of these, seven did not transfer the amplification products to cultivars Jalo EEP 558 and Small White. Of the 98 primers that transferred the amplification products, 44 presented polymorphic bands with 100 to 200 base pairs. Through the adjustment test of Mendelian segregation, considering the Bonferroni correction, two SSR markers were discarded so 42 markers were considered for mapping. Figure 2 presents the established linkage map and Table 2 the distances between markers of the map. The map was constructed with 24 markers, arranged in eight linkage groups (c1 to c8) with a mean length of 51.01 cM and a mean marker distance of 25.01 cM. The map comprised 400.1 cM, corresponding to 23.26% of the common bean genome, considering the map obtained by Blair et al. (2003).

The high minimum LOD value allowed the identification of linkage groups with marker intervals below 40 cM only. According to Lee (1995), the useful interval for a preliminary QTL identification lies between 15 and 20 cM or a little higher. This points to the need for a greater saturation in the linkage map to amplify the possibilities of using these markers in molecular marker-assisted selection.

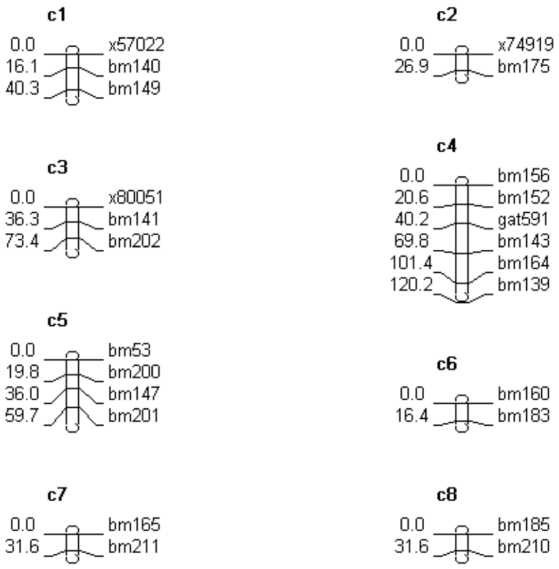

Figure 2. Linkage map using SSR markers and F_2 generation of crossing Jalo EEP 558 x Small White

Multiple linear regression analyses showed that in the individual environments markers explained about 25% of the phenotypic variation, since the R^2 of the obtained models varied from 17.92% under the conditions of Lavras in the dry period of 2003 to 31.02% for the conditions of Lambari in the dry period of 2003. Among the markers that explained phenotypic variation, BM146 and BM210 stood out in evaluations of individual environments. Marker BM146 explained alone 9.69% in the dry period of 2002 in Lavras and marker BM210 8.13% in the dry period of 2003

Table 2. Linkage groups, markers and distances between adjacent markers given by the Kosambi mapping function of the linkage map

Linkage group	Marker	Distance	Linkage group	Marker	Distance
C1	X57022		C5	BM53	
	BM140	16.1		BM200	19.8
	BM149	24.2		BM146	16.2
C2	X74919			BM201	23.7
	BM175	26.9	C6	BM160	
C3	X80051			BM183	16.4
	BM141	36.3	C7	BM165	
	BM202	37.1		BM211	31.6
C4	BM156		C8	BM185	
	BM152	20.6		BM210	31.6
	GAT591	19.6			
	BM143	29.6			
	BM164	31.6			
	BM139	18.8			

in Lambari. Marker BM165 was also constant in the evaluations realized in the dry period 2002, explaining 7.25% of the phenotypic variation in the model obtained for the conditions of Lambari.

A series of other markers were identified as associated with angular leaf spot tolerance under isolated environmental conditions, some of which, when inserted into the model, led to considerable R^2 increases. The indication of these markers as potential for assisted selection, even when the objective of the program is specific adaptation, would however be untimely and requires further evaluations.

When considering the multiple linear regression analyses realized with the means used in the joint analyses as dependent variable, we note that the percentage of the phenotypic variation explained by the models was high. It varied from 19.72%, when using means of the dry period 2003 in Lavras and Lambari, to 29.57% when using means of the evaluations of the dry period of 2002. In most of the multiple linear regression analyses with the joint means, markers BM146 and BM210 were the ones that explained most of the phenotypic variation. Despite studies on control of the reaction to angular leaf spot in common bean are not yet conclusive, these results agree with the digenic inheritance with modifiers postulated by Bett and Michaels (1995) and by Rezende et al. (1999).

In the conditions of Lavras, it was also possible to verify the contribution of marker BM200 which explained alone about 10% of the phenotypic variation. In the conditions of Lambari, however marker BM210 explained alone 10.46% of the phenotypic variation. In the dry period of 2002, besides markers BM146 and BM210, marker BM165 stood out, explaining alone around 9% of the phenotypic variation. In the conditions of the dry period 2003 however, only a small percentage of the variation was explained when other markers were inserted into the model that already contained the markers BM210 and BM146. When considering the means of the four evaluations, once more it was possible to observe the great contribution of markers BM210 and BM146 to the model, indicating they are associated to stable QTL. However, the control of angular leaf spot resistance and QTL associated to marker BM165 are more influenced by specific environmental conditions. One must consider that the manifestation of tolerance to angular leaf spot in common bean can be a consequence of genetic differences between the common bean cultivars, races of angular leaf spot and environmental conditions (Melo et al. 2002).

Table 3 presents the identified QTL, their positions in the linkage groups and their LOD scores. The individual analyses showed that under the dry period conditions of 2002 in Lavras as much as in Lambari, QTL were identified in linkage group c5 associated to the reaction to angular leaf spot, although in the conditions of Lavras the QTL is within the interval delimited by the markers BM53 and BM146. Under the conditions of Lambari however, the QTL is restricted to the interval between the markers BM200 and BM146. In the dry period of 2003 no significant QTL associated to the grade of reaction to angular leaf spot were found in either conditions of Lavras or Lambari.

Table 3. QTL identified for the reaction to angular leaf spot by the method of composite interval mapping and their positions in the linkage map

Environment	Linkage group	Position in the linkage group	LOD
Lavras – dry period of 2002	C5	0.2381	4.70
Lavras – dry period of 2002 and 2003	C5	0.2781	3.60
Lambari – dry period of 2002 and 2003	C5	0.2981	3.64
	C8	0.2201	3.29
Lavras and Lambari – dry periods of 2002 and 2003	C5	0.2781	5.56
Lavras and Lambari – dry periods of 2002 and 2003	C5	0.2781	2.15

The treatment means obtained in Lavras and Lambari in the dry period of 2002 confirmed the QTL localized in the interval between markers BM53 and BM146. Although these QTL had had no expression in harvest 2003, QTL were identified associated to the grade of reaction to angular leaf spot when considering the joint analyses of Lavras in the dry periods of 2002 and 2003 and Lambari in the dry periods of 2002 and 2003. In the case of Lavras, this QTL was present in the interval between markers BM53 and BM146, while under the conditions of Lambari the QTL was present in the interval between BM200 and BM146.

In the joint analysis in that considered the evaluations realized in the dry period of 2002 and 2003 in Lambari, one QTL was identified in linkage group c8, close to marker BM210. This QTL was only identified in the conditions of Lambari and, considering the means of the dry period of two consecutive years, it may represent the expression of genes that provide tolerance to local races of the pathogen that would be associated to these environmental conditions.

Regarding the joint analyses that involved the four evaluations the effect of a QTL localized in the linkage

group c5 between BM53 and BM146 was observed on the expression of reaction to angular leaf spot. Comparing the analyses of QTL identification by multiple linear regression and composite interval mapping it was possible to verify that the strong association with the reaction to angular leaf spot of marker BM210 in the individual analyses in Lambari by the method of multiple regression could only be identified in the joint analyses by the method of composite interval mapping.

The results obtained with composite interval mapping for QTL identification are preliminary since the map obtained with SSR markers was little saturated and comprises a smaller number of linkage groups than the base number of chromosomes in common bean.

The presence of two major genes controlling the trait agrees with the supposition of digenic inheritance of Bett and Michaels (1995) and Rezende et al. (1999). The assumption that besides the vertical, horizontal inheritance is involved in the control of angular leaf spot is also worth considering due to the identification of markers explaining a low percentage of phenotypic variation.

Mapeamento de QTLs para reação à mancha angular do feijoeiro por meio de marcadores microssatélites

RESUMO - *A identificação de QTLs é o passo inicial para aplicação da seleção assistida por marcadores moleculares no melhoramento. O feijoeiro conta com mapa molecular, QTLs identificados e, recentemente, com marcadores SSR. O objetivo desse trabalho foi identificar QTLs usando marcadores SSR em famílias segregantes do cruzamento das cultivares Jalo EEP 558 e Small White. Essas famílias apresentaram diferenças significativas para reação à mancha angular, sendo obtidas elevadas estimativas de herdabilidade. O efeito do ambiente e da interação genótipos por ambientes influenciaram a expressão da reação à mancha angular. Foi obtido um mapa com 400,1 cM por meio do uso de 24 marcadores, dispostos em oito grupos de ligação com comprimento médio de 50,01 cM e distância média entre marcadores adjacentes de 25,01 cM. Foram identificados QTLs estáveis e associados a altos percentuais da variação fenotípica envolvidos na expressão da reação à mancha angular, destacando-se os marcadores BM210 e BM146.*

Palavras-chave: *Phaseolus vulgaris*, *Phaeoisariopsis griseola*, SSR.

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