Genetic map of the common bean using a breeding population derived from the Mesoamerican gene pool

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ABSTRACT - The mapping population consisted of 94 F₂ generation plants derived from a cross between the CNFC 7812 and CNFC 8056 lines, with different protein contents, 24% and 19% respectively. Seven hundred and fifty-two molecular markers were tested among the parents and four individuals from the segregant population. A total of 101 loci were used to develop the genetic map. The polymorphism rate was 8.3% and 23.2% for the microsatellite and RAPD markers, respectively. The sizes of the linkage groups ranged from 6.7 to 139.0 cM, presenting a mean of 49.4 ± 36.8. The map length was 840.7 cM and the mean group length was 45.9 cM. The average distance between the framework loci was 16.1 cM. This map was compared with international reference bean maps and results were discussed. The construction of the genetic map from parents of the same center of origin and the commercial grain type were discussed.

Key words: microsatellites, RAPD, breeding program, Mesoamerican gene pools, Phaseolus vulgaris L.

INTRODUCTION

The common bean is the main legume used in human nutrition and is very important, economically and socially. It is a key element of a protein source in the diet in the world population, especially in Latin America and African countries (Broughton et al. 2003). The common bean (Phaseolus vulgaris L., 2n = 2x = 22) is a self-pollinating, diploid species with 0.66 picograms/DNA haploid genome, that is equivalent to 450 to 650 million base pairs (Mb) haploid genome⁻¹ (Arumuganathan and Earle 1991, McClean et al. 2004). Molecular markers have been an important tool to characterize and determine genetic diversity among common bean cultivars (Blair et al. 2006, Benchimol et al. 2007, Hanai et al. 2007), to construct genetic maps and to search for genome regions associated with genes of interest (QTLs) (Miklas et al. 2006, Miklas 2007, Rodriguez-Suarez et al. 2007).

The first common bean genetic maps developed from molecular markers was performed in the 1990s (Vallejos and Chase 1991). Vallejos et al. (1992) constructed the first genetic map for the common bean, based on morphological, RFLP, isoenzymes and RAPD markers, called the Florida map, from the cross between a line of Mesoamerican origin and Andean origin. Nodari et al. (1993) developed the genetic map called the Davis map obtained from the F₂ generation derived from the cross between the BAT 93 (CIAT) and Jalo EEP558 (Brazilian) genotypes using RFLP markers. Later,
Adam-Blondon et al. (1994) constructed the Paris map using RFLP, RAPD and morphological markers, from the common bean backcross families (Ms8E02 x Corel). Freyre et al. (1998) constructed an integrated linkage map that established the correspondence between the linkage groups of the previously published maps (Davis, Florida and Paris maps). Pedrosa et al. (2003) used the in situ fluorescence hybridization analysis to connect the chromosomes and the linkage map developed by Vallejos et al. (1992). More recently, genetic maps have been developed for the common bean using microsatellite markers (Blair et al. 2003, Grisi et al. 2007).

Most of the genetic maps constructed for the common bean were obtained from crosses between lines derived from different centers of origin and/or different genetic sets (Vallejos et al.1992, Nodari et al. 1993, Freyre et al. 1998, Blair et al. 2003, Grisi et al. 2007). This strategy is interesting, because it permits a maximization of the polymorphisms generated in the crosses due the genetic divergence existent among parents. However, the validation of the QTLs detected in this genetic background in other breeding populations has not yet been studied. Furthermore, there is little chance of obtaining lines for immediate use in the breeding programs derived from crosses which involve very divergent parents. These aspects hinder the fast use of these genetic maps in common bean breeding programs.

An alternative would be the construction of the common bean genetic maps using parents derived from the same gene pool. The objective of this study was to construct a linkage map in the common bean using microsatellite and RAPD markers from population mapping derived from Mesoamerican lines.

**MATERIAL AND METHODS**

**Plant material and DNA extraction**

The mapping population consisted of 94 F2 individuals obtained from the cross between the CNFC 7812 and CNFC 8056 lines (CNFC = Carioca Bean National Center) from the Embrapa Rice and Bean breeding program (Santo Antonio de Goiás, Goiás, Brazil). These lines have the following genealogy: CNFC 7812 = BZ3836-1/FE9166/AN910523 and CNFC 8056 = MA720943/CB733860/AN512545/RH20-414. Both lines belong to the Mesoamerican center of origin, are of the carioca grain type (same commercial group), are high yield, have semi-prostrate plant architecture and an intermediate reaction to angular spot caused by *Pseudocercospora griseola*, anthracnoses caused by *Colletotrichum lindemuthianum* and bean golden mosaic virus. The CNFC 7812 and CNFC 8056 parents have different protein contents, with 24% and 19%, respectively.

Approximately 250 mg of leaf tissue from the parents and the 94 F2 individuals were collected for genomic DNA extraction using the CTAB method proposed by Hoisington et al. (1994). DNA was quantified by comparing the fluorescence intensities of the samples with those of DNA standards in ethidium bromide stained gels (10 mg mL-1) under UV light. The electrophoresis (3 V cm⁻¹) was carried out on 0.8% agarose gels.

**Molecular analysis**

**Microsatellite markers**

A total of 493 pairs of microsatellite primers were used to assess polymorphism, 403 of which were derived from genomic libraries (Gaitán-Solís et al. 2002, Buso et al. 2006, Benchimol et al. 2007) and 90 were obtained from express sequence tags (ESTs) (Hanai et al. 2007).

The amplification reactions of the microsatellite were carried out in a PTC-100 Thermal Cycler (MJ Research) and the PCR conditions were used as reported in literature (Gaitán-Solís et al. 2002, Buso et al. 2006, Benchimol et al. 2007, Hanai et al. 2007). The amplification reactions were performed in a final volume of 15 μL containing 15 ng DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl (pH 8.0), 1.5 mM MgCl2, 0.8 mM dNTPs, 0.28 μM primers (forward and reverse) and 1U Taq polymerase (Invitrogen, CA).

Reaction products were mixed with a 5 μL solution of formamide dye (98% formamide, 10 mmol L⁻¹ EDTA pH 8.0, bromophenol blue 0.002%, xylene cyanol 0.002%). The mixture was denatured at 95 °C for 5 min, and 7 μL were loaded on the wells. Previously warmed 1× TBE buffer (100 mmol L⁻¹ Tris, 100 mmol L⁻¹ boric acid, 2 mmol L⁻¹ EDTA) was used to prerun 0.5 mm-thick 6% polyacrylamide denaturing gels (6% acrylamide/bisacrylamide (19:1) and 7.5 mol L⁻¹ urea gel) in a BioRad (Hercules, Calif.) 38 cm × 50 cm sequencing apparatus for 1 h at 80 W. Gels were run for 3 h at 85 W. The DNA was visualized by silver staining as described in detail by Creste et al. (2001).
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**RAPD markers**

A total of 259 10-base random primers (kits A, AA, AB, AC, AE, AF, AJ, B, C, D, E, F, H, I, J, L, N, O, P, R, Z and W from Operon Technologies, Alameda, CA) were used for assessing the polymorphism. RAPD reactions were performed in a final volume of 15 μL containing 10 mM Tris-HCl (pH 8.3), 2.8 mM MgCl₂, 50 mM KCl, 0.2 mM each dNTPs, 2 μM primer, 15 ng template DNA, and 1.0 U Taq DNA polymerase. Controls were prepared replacing DNA with water. The RAPD reactions were carried out in My Cycler (BioRad) thermocycler, consisting of preliminary denaturation (5 min at 94 °C), 40 cycles of denaturation (1 min at 94 °C), primer annealing (1 min at 35 °C), and extension (2 min at 72 °C), followed by a final extension at 72°C for 6 min. Amplified fragments were separated by electrophoresis on 1.4% agarose, 1x TBE (89 mM Trisborate, 2 mM EDTA (pH 8.3) gels run at 3 V cm⁻¹. DNA bands were visualized under UV light after an ethidium bromide stained gel.

To minimize experimental artifacts in RAPD analysis, we used two DNA concentrations (15 and 30 ng). Markers that did not consistently amplify or could not be scored reliably across the two DNA concentrations were not analyzed further. The gels were examined by two different evaluators.

**Segregation deviations and linkage analysis**

Segregation ratios were tested by χ² analysis using the False Discovery Rate (FDR) criterion (Benjamini and Hochberg 1995) to determine the significance level. The SSR and RAPD markers that deviated from the expected segregation were maintained in the linkage analysis.

The linkage analyses were established using the Joinmap program version 3.0® (Van Ooijen and Voorrips 2001). The mapping was carried out using the F₂ procedure, recommended by the program to construct linkage maps from an F₂ population resulting from the self pollination of plants in the F₁ generation derived from crossing two completely homozygote parents. The linkage groups were established using the LOD grouping command with a minimum LOD score of 3. For each linkage group, marker orders were established in two steps. The first step, a maximum recombination frequency of 0.30 and a minimum LOD score of 2, and values of jump = 5 and ripple = 3 were adopted as the limiting criteria. According to these criteria the map framework was created, which was indicated by the fixed order command. In the second step, new markers were allocated to the framework using the recombination frequency criteria and lower LOD scores than in the first stage, but the values attributed to jump and ripple were maintained from the previous step. The recombination fractions were converted into map distance values by the Kosambi function (Kosambi 1944). The final version of the linkage map was drawn using the MapChart program (Voorrips 2002).

A comparative analysis was carried out based on the common SSR markers between the obtained map in the present work and the other three maps previously developed for the common bean using the populations: DOR364 x G19833 (Blair et al. 2003), BAT 93 x Jalo EEP558 (Grisi et al. 2007) and Cerinza x G24404 (Blair et al. 2006). The SSRs in common between the maps were evaluated in terms of linkage conversion and the order of the linkage groups. The maps described in the literature establish relationships between the linkage groups and the common bean chromosomes, and the nomenclature adopted in the chromosome denomination was proposed by Pedrosa-Harand et al. (2008).

**RESULTS AND DISCUSSION**

**Polymorphism survey**

A total of 493 microsatellite loci were tested in the primer selection. After optimizing the PCR conditions, 17 (3.4%) did not amplify, 41 (8.3%) resulted in a multiple band pattern and 435 (88.2%) amplified consistent products. Of these, 41 (9.4%) were polymorphic among the parents and 394 (90.6%) were monomorphic. Of the 41 polymorphic loci, 32 (78%) were obtained from genomic libraries and 9 (21%) from gene-based libraries. Blair et al. (2003) observed that the microsatellite markers from gene-based libraries (46.2%) were less polymorphic compared to those from genomic libraries (64.2%). Nevertheless, Hanai et al. (2007) assessed common bean lines from EST- and genomic SSR and verified fairly similar values in the average of allele per locus and the mean PIC value (Polymorphic Information Content). According to Varshney et al. (2005), microsatellites derived from EST libraries tend to be less polymorphic when compared to those from genomic libraries, due to a greater conservation of the DNA sequence of the transcribed and translated regions of the genome. However, Morgante et al. (2002) proposed that microsatellites from EST sequences located in
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transcribed regions, but not translated, tended to present greater allele polymorphism than genomic SSRs.

Out of the 259 RAPD primers analyzed, 239 (92.3%) showed a clear and reproducible band profile, and 20 (7.7%) did not amplify any fragment. Of the primers that amplified fragments, 18.4% (44 primers) showed at least one polymorphic locus and 81.6% (195) were monomorphic. The polymorphic primers generated 332 reproducible amplification products in an average of 7.5 bands per primer. The average length of the RAPD fragments was 1027 pb, showing a variation in maximum and minimum size of 1900 pb and 200 pb, respectively.

The polymorphic rates detected by RAPD vary greatly in the common bean. Alzate-Martin et al. (2001) studied the genetic variability of the elite common bean genotypes via RAPD markers and found an average number of 2.6 polymorphic bands primer\(^{-1}\). Galván et al. (2006) analyzed the genetic diversity of Argentinean common bean genotypes and observed that the number of polymorphic loci was 4.2 primer\(^{-1}\) in the current study, it is likely that the low number of polymorphic primer\(^{-1}\) loci occurred because of the genetic proximity of the lines used in the cross. Although the reproducibility of the RAPD markers is low, some authors have shown that the RAPD can be reliable molecular markers, providing care is taken to control variations in the components of the PCR reaction (Carneiro et al. 2002). In our study, we combined a high quality DNA template preparation, the use of two concentrations DNA and genotyping by two evaluators only from the most intense fragments. By doing so, it was possible to achieve reproducible amplification products, thus minimizing genotyping errors.

The level of polymorphism is directly related to the genetic diversity present in the population under study. In the present study, the low polymorphism obtained (13.4%, 101/752) may be attributed to the narrow genetic base of the cultivated plants, and in this specific case to the parents that belonged to the same center of origin (Mesoamerican) and presented the same grain type (carioca). Blair et al. (2006) observed that the average level of polymorphism was higher in the intergenepool (Andean x Mesoamerican) combinations (59.7%) than within-genepool (37.9%) combinations based in microsatellite markers. Among the within-genepool parental combinations, comparisons between Andean genotypes had higher polymorphism (53.0%) on average than comparisons between Mesoamerican genotypes (33.4%). The authors verified that the polymorphism was even lower for the parental comparison between two parents of the same grain color and the same sub-race of the Mesoamerica race. In this sense, our work corroborates the results obtained by Blair et al. (2006). Therefore, polymorphism increases as the population presents greater genetic diversity, and probably one of the reasons most of the genetic maps published (Freyre et al. 1998, Blair et al. 2003, Blair et al. 2006, Grisi 2006) used parents from different centers of origin (Andean x Mesoamerican).

**Linkage map**

The summary of the main characteristics of the linkage map can be found in Table 1. A total of 69 loci (68.3%) were mapped and distributed along 17 linkage groups (Figure 1). Of the total markers mapped, 43 (62.3%) were allocated on the framework map. Thirty two markers (31.7%) were not attributed to any one of the linkage groups (unlinked markers): 25 RAPD markers (W06_1594, B10_1050, H01_545, AB02_1767, AA19_1050, H07_1169, J09_810, F06_704, R07_584, E12_1235, AJ18_960, J18_1430, B10_1076, H12_1262, H01_930, AE20_748, J15_1288, H02_1448, F06_638, F06_1115, E06_624, J09_996, AF07_1262, AE17_1368 and H02_1315) and 7 SSRs (PvBr81, PvBR114, BM 141, BM187, BM154, PvM78 and PvM49) (five genomic and two gene-based SSRs).

Approximately 25% of the intervals in the linkage group were greater than 25 cM with 42.3 cM as the greatest and 0.3 cM as the smallest distance detected. Linkage group 1 presented the greatest number of markers (11), which were well distributed with an average

### Table 1. Description of the linkage map of *Phaseolus vulgaris* L., generated from the population *F*\(_2\) obtained crossing between the lines CNFC 7812 and CNFC 8056, using RAPD and microsatellite markers

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Map</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of markers (SSR/RAPD)</td>
<td>101(41/60)</td>
</tr>
<tr>
<td>No. of linkage groups</td>
<td>17</td>
</tr>
<tr>
<td>No. of distorted markers (SSR/RAPD)</td>
<td>15(3/12)</td>
</tr>
<tr>
<td>No. of unlinked markers (SSR/RAPD)</td>
<td>32(7/25)</td>
</tr>
<tr>
<td>Map length(^a) (cM)</td>
<td>840.7</td>
</tr>
<tr>
<td>Marker density (cM/marker)</td>
<td>16.1</td>
</tr>
<tr>
<td>Maximum/minimum distance (cM)</td>
<td>42.3/0.3</td>
</tr>
<tr>
<td>Maximum/minimum length of the groups (cM)</td>
<td>139.0/6.7</td>
</tr>
<tr>
<td>Average Length of groups (cM)</td>
<td>49.4</td>
</tr>
</tbody>
</table>

\(^a\)Corresponds to the sum of group sizes, established with Joinmap
distance of 13.9 cM and in the formation of a cluster, with a distance of 0.3 cM (D06_637, A08_1460). Groups 16 and 17 where the smallest linkage groups (6.7 cM), presenting only two linkage markers, that were established by the framework criteria. The RAPD and microsatellite markers were not uniformly distributed in the linkage groups (Figure 1). Approximately 46.4% of the marker loci were confined to groups 1, 2, 3 and 4, which jointly accounted for 49.2% of the total map length.

Nine of the 15 markers that presented expected Mendelian segregation distortion were not placed in the linkage groups. Six markers (B08_1714, AF15_1102, PvBR29, AA18_1634, W06_836 and U18791) were mapped along six of the linkage groups (Groups 2, 5, 8, 11, 13 and 15). No clusters were observed of the distorted loci in the preferential regions in the linkage groups.

Eighteen microsatellite markers were common to the present map and to the maps published by Blair et al. (2003), Blair et al. (2006) and/or Grisi et al. (2007). The PvBR11, PvBR61, PvBR93, PvBR35, PvBR54, PvBR235, PvBR107, PvBR46, PvBR87, PvBR269 and PvBR213 loci were observed exclusively between the present map and the map developed by Grisi et al. (2007). The BM 189 and BM 205 were exclusive between the present map and those developed by Blair et al. (2003) and Blair et al. (2006). The BM 152, BM 211, BM 170, BM 183 and BM143 were found in the present map and those proposed by Grisi et al. (2007) and Blair et al. (2003), Blair et al. (2006).

Comparative mapping of the three previously published linkage maps (Blair et al. 2003, Blair et al. 2006, Grisi et al. 2007) allowed us to relate the three linkage groups obtained in this study with the chromosomes of the *P. vulgaris* species. Linkage group 1 (present map) was related to chromosome 2 through markers PvBR11, BM 152, BM 143 and PvBR213; linkage group 8 (present map) was related to chromosome 5 through markers PvBR61 and PvBR93; linkage group 6 (present map) was related to chromosome 7 through markers PvBR35 and PvBR269.

In the present study, the total distance of the map was estimated as 840.7 cM, approximately 68.6% of the value of 1226 cM of the core linkage map established by Freyre et al. (1998), and similar to the value of 869.5 cM obtained by Blair et al. (2006), who used 84 markers (84 microsatellite, one SCAR, two morphological and the phaseolin protein). Initially the genetic map developed in the present study was based on microsatellite marker analysis to construct the linkage groups. However, because of the low polymorphisms detected by the SSR among the parents, RAPD markers were chosen to diversify the marker types. It was considered that because almost all of the SSR markers used in the present study came from enriched genomic

**Figure 1.** Genetic linkage map of common bean based on markers microsatellite and RAPD, developed from the analysis of segregation of the population derived from the cross between the lines CNFC 7812 and CNFC 8056. Molecular markers in bold made up the framework. The distorted loci (P <0.05), based on the FDR correction criterion, were indicated with an asterisk.
libraries, it is possible that some loci may have shared the same region of the genome, causing redundancies in the genetic information. In addition, the RAPD markers permit the exploration of genome regions that may be different from those scanned by the microsatellite markers, giving wider coverage of the possible polymorphism points present in the parents. The RAPD polymorphic primer rate identified was approximately twice that found in the SSR markers. In the present study, the RAPD loci were useful to amplify the number of points on the genetic map. Meanwhile, the nature of RAPD polymorphism is binary, that is, they are dominant, which hinders the use of this set of markers in homology and integration studies with previously published common bean genetic maps.

The number of linkage groups presented in this study was greater than the haploid chromosome numbers of *P. vulgaris* (n = 11). However, many of the small linkage groups could be joined with other linkage groups when additional markers were added to the map. The increase in the number of markers could help to improve the resolution of the linkage map, reducing the number of linkage groups to the haploid number of the species. Another alternative would be to increase the sample size of the individuals of the segregated population, which would amplify the number of samples of meiotic events allowing for the connection of some of the linkage groups. The excess number of linkage groups compared to the haploid number of the species suggests that some areas of the *P. vulgaris* genome have not been detected with the set of markers used in the present study.

The genetic map construction is based on greater polymorphism among parents to elaborate saturated maps. The parents chosen for mapping should be contrasting, because the number of marks on the map represents the number of contrasting points found in the parents (Liu 1998). In Brazilian breeding programs, obtaining modern common bean cultivars prioritizes crosses among elite genotypes, mainly among materials from the same center of origin (Mesoamerican x Mesoamerican or Andean x Andean). Crosses resulting from different genetic sets can bring undesirable genetic linkage that increases the probability of unfavorable alleles occurring, which would increase the time required to obtain common bean cultivars. In general, the main international common bean maps were constructed from genotypes belonging to different centers of origin (Mesoamerican x Andean) (Vallejos et al. 1992, Nodari et al. 1993, Freyre et al. 1998, Blair et al. 2003, Blair et al. 2006, Grisi et al. 2007) where efficiency in obtaining commercial lines, even when orientated by marker assisted selections, is low and time-consuming. For these maps, the loci of interest found (QTLs) should be tested in the segregant populations of the breeding program, where the validation of these genome regions will not be guaranteed, hindering the use of these loci in the assisted selection process.

However, international common bean maps have shown high polymorphic levels permitting their wide saturation. The polymorphic rate detected in the present map was approximately 4.2 times smaller (13.4% versus 56%) than that found on the map developed by Blair et al. (2003). Thus the construction of common bean genetic maps using genetically close genotypes will result in a greater effort of development and genotyping of molecular markers than those maps constructed from genotypes from different centers of origin.

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Mapa genético do feijoeiro comum utilizando população de melhoramento derivada do pool gênico Mesoamericano

RESUMO - A população para o mapeamento foi composta por 94 plantas da geração F₂ derivadas do cruzamento entre as linhagens CNFC 7812 e CNFC 8056, contrastantes para o teor de proteína, com respectivamente 24 e 19%. Setecentos e cinquenta e dois marcadores moleculares foram testado entre os genitores e quatro indivíduos da população segregante. Um total de 101 locos foram usados para construção do mapa genético de ligação. A taxa de polimorfismo foi de 8,3% e 23,2% para os marcadores RAPD e microsatélites, respectivamente. O tamanho dos grupos de ligação variaram de 6,7 a 139,0 cM, apresentando uma média de 49,4 ± 36,8. O comprimento do mapa foi de 840,7 cM e o comprimento médio dos grupos foi de 45,9 cM, com uma distância média entre os marcadores de 16,1 cM. Este mapa foi comparado com mapas referência internacional em feijoeiro e os resultados foram discutidos. A construção de mapas genéticos em feijoeiro a partir de genitores do mesmo centro de origem e tipo de grão comercial foi discutida.

Palavras chave: microsatelites, RAPD, programa de melhoramento, marcadores moleculares, pool gênico Mesoamericano.

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