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ORIGINAL PAPER

High macro-collinearity between lima bean (*Phaseolus lunatus* L.) and the common bean (*P. vulgaris* L.) as revealed by comparative cytogenetic mapping

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Abstract Common bean (*P. vulgaris*) and lima bean (*P. lunatus*) are the most important crop species from the genus *Phaseolus*. Both species have the same chromosome number ($2n = 22$) and previous cytogenetic mapping of BAC clones suggested conserved synteny. Nevertheless, karyotype differences were observed, suggesting structural rearrangements. In this study, comparative cytogenetic maps for chromosomes 3, 4 and 7 were built and the collinearity between the common bean and lima bean chromosomes was investigated. Thirty-two markers (30 BACs and 2 bacteriophages) from *P. vulgaris* were hybridized in situ on mitotic chromosomes from *P. lunatus*. Nine BACs revealed a repetitive DNA pattern with pericentromeric distribution and 23 markers showed unique signals. Nine of these markers were mapped on chromosome 3, eight on chromosome 4 and six on chromosome 7. The order and position of all analyzed BACs were similar between the two species, indicating a high level of macro-collinearity. Thus, although few inversions have probably altered centromere position in other chromosomes, the main karyotypic differences were associated with the repetitive DNA fraction.

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Introduction

Lima bean (*Phaseolus lunatus* L.) is an important source of protein for the human diet, especially in warmer and drier regions where common bean does not grow well (Maquet et al. 1999). This species is very similar to the common bean (*P. vulgaris* L.) in that both species share similar wild distribution and domestication in two independent regions: Andean and Mesoamerican (Debouck et al. 1987; Fofana et al. 1999; Gepts 1998; Gutiérrez-Salgado et al. 1995; Lioi 1996; Maquet et al. 1999). Both species have $2n = 22$, similar genome sizes (637 Mbp in *P. vulgaris* and 622 Mbp in *P. lunatus*) and a predominance of metacentric and submetacentric chromosomes (Arumuganathan and Earle 1991; Moscone et al. 1999; Schweizer and Ambros 1979; Zheng et al. 1991). Differences in chromosome morphology have led to the suggestion that rearrangements such as inversions, duplications and translocations have occurred in the genus (Mercado-Ruaro and Delgado-Salinas 2000). However, it is unclear whether these changes have indeed played a significant role in karyotype evolution in this group.

The distribution of heterochromatin, detected by C-banding and fluorochrome staining, reinforced the karyotype similarity in the genus, with blocks in the pericentromeric regions of most chromosomes (Mok and Mok 1976; Zheng et al. 1991, 1993), except for *P. lunatus*, for which Moscone et al. (1999) described blocks in the terminal regions. The location of the 5S and 45S rRNA genes in four cultivated species allowed a more detailed comparison of karyotypes; however, these two markers did not detect clear homologies between *P. vulgaris* and *P. lunatus* (Moscone et al. 1999).

Several tools have been developed to support common bean breeding, including genetic maps (Adam-Blodon et al.

1994; Freyre et al. 1998; Nodari et al. 1993; Vallejos et al. 1992) and bacterial artificial chromosome (BAC) libraries (Kami et al. 2006). These tools have allowed the establishment of a cytogenetic-based physical map for this species, integrated to its genetic map (Fonsêca et al. 2010). For chromosomes 3, 4 and 7, a higher number of BAC markers were available and genetic and physical distances could be compared in more detail (Pedrosa-Harand et al. 2009).

Recently, Bonifácio et al. (2012) developed a comparative cytogenetic map of *P. lunatus*, establishing the chromosome homologies to *P. vulgaris* and demonstrating a conservation of synteny between both genomes. However, the low number of markers per chromosome did not make it possible to analyze the degree of collinearity between the two species in more detail. In the present study, markers from chromosome 3, 4 and 7 of the common bean were mapped by FISH to the chromosomes of the lima bean to analyze if collinearity is also maintained between these genomes.

Materials and methods

Plant material and chromosome preparation

Seeds from *P. lunatus* accession 'Vermelhinha' (GL0135) were obtained from the germplasm bank of Embrapa Arroz e Feijão (Brazil). Somatic chromosome preparation, selection of slides and destaining for FISH were performed following Cabral et al. (2006), except that root tips were pre-treated with 2 mM 8- hydroxyquinoline (Sigma-Aldrich) at 12 °C for 18 h and that the digested material was washed in distilled water and kept in water overnight at 4 °C before preparing slides.

DNA probes

BAC clones from the common bean (genotype BAT93) *Hin*dIII genomic library (Kami et al. 2006) were used. These BACs were previously selected by screening the BAC library with RFLP clones (*Bng*s) mapped to linkage groups A, B and C (Vallejos et al. 1992) and were later mapped to the common bean chromosomes 3, 4 and 7 by Pedrosa-Harand et al. (2009). Additionally, two λ bacteriophages also mapped to chromosome 4 were used (Pedrosa-Harand et al. 2009). All genomic clones were labeled by nick translation (Roche Diagnostics) with Cy3-dUTP (GE Healthcare).

Fluorescence in situ hybridization (FISH)

The in situ hybridization procedure was performed according to Jiang et al. (1995) with some modifications. Chromosomes were denatured in 70 % formamide in 2× SSC for 1.5 min at 75 °C and dehydrated in an alcohol series. The hybridization mixtures consisted of 50 % (v/v) formamide, 10 % (w/v) dextran sulfate, 2× SSC and 2–5 µl of probe. *Phaseolus vulgaris* C_0t-100 fraction was added in 20- to 100-fold excess to the hybridization mix, according to what was necessary for this species, to block repetitive sequences. The mixture was denatured for 10 min at 75 °C, added to the chromosome preparations and hybridized for 18–36 h at 37 °C. The stringent wash was performed with 0.1× SSC at 42 °C. Preparations were counterstained and mounted with 2 µg/ml DAPI (Sigma-Aldrich) in Vectashield (Vector). After analysis, the preparations were destained in ethanol/acetic acid 3:1 (v/v), dehydrated in absolute ethanol for 2–18 h and re-hybridized with a different probe.

Data analysis

Photographs were taken with a Cohu CCD camera attached to a Leica DMLB microscope and using the Leica QFISH software. Image adjustments and chromosome measurements were done with Adobe Photoshop 7.0 software (Adobe Systems Inc.). Idiograms were constructed using Macromedia Flash MX 2004 software (Macromedia Inc) and chromosomes were named according to the nomenclature of the corresponding linkage groups proposed by Freyre et al. (1998) and defined by Pedrosa-Harand et al. (2008) as the standard nomenclature for common bean chromosomes. The pairwise *T* Student test was applied for comparing the positions of markers, with 5 % of probability, using the R package software.

Results

A total of 32 clones (nine BACs from chromosome 3, nine BACs and two bacteriophages from chromosome 4 and 12 BACs from chromosome 7) from *P. vulgaris* (Pedrosa-Harand et al. 2009) were used for cytogenetic mapping in *P. lunatus*. Chromosome 3 was submetacentric with an arm ratio of 2.12 and showed a $DAPI^+$ band after FISH in the proximal region of the long arm (Fig. 1d, g). Chromosomes 4 and 7 were metacentric with arm ratios of 1.18 and 1.37, respectively. Chromosome 3 was the largest of the three chromosomes, with chromosomes 7 and 4 having relative lengths of 0.91 and 0.84 compared to chromosome 3, respectively.

Three general patterns of labeling of the chromosome complement were observed (Tables 1, 2). Seven BACs showed a dispersed hybridization pattern on all chromosomes (Fig. 1a, b). A similar pericentromeric pattern was observed for two other BACs, which labeled the regions

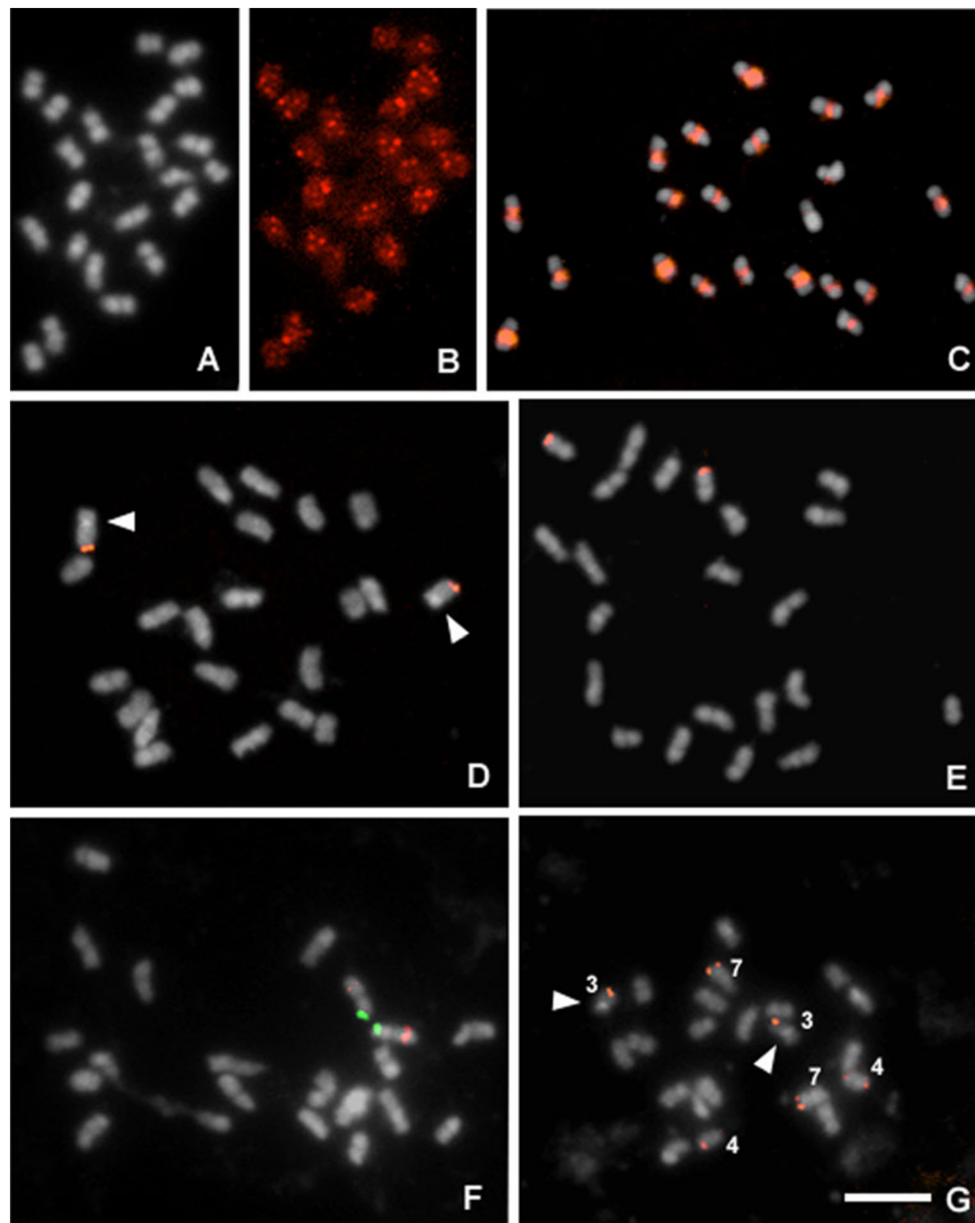

Fig. 1 In situ hybridization on mitotic chromosomes of *P. lunatus*. **a**–**b** dispersed repetitive DNA revealed with BAC 20F21 probe, **c** pericentromeric pattern from BAC 53G1, **d** unique signal observed on chromosome 3 with BAC 95L12, **e** unique signal of BAC 221J10 on chromosome 4, **f** FISH mapping of two clones (BAC 86I17 in *green* and BAC 33M20 in *red*) on chromosome 7, **g** multiFISH mapping of three chromosomes. Chromosomes are counterstained with DAPI and visualized in *gray*. The DAPI^{+} banding pattern on chromosome 3 are indicated by *arrowheads*. The *bar* in **g** represents 5 µm (color figure online)

around the centromeres of all chromosomes (Fig. 1c; Table 1). For these nine BACs, C_0t-100 repetitive fraction of *P. vulgaris* DNA was used as a blocker; however, although the blocker had decreased repetitive signals, no unique signal was observed. Differences were observed between *P. vulgaris* and *P. lunatus*, such as for BACs 26B20 and 267K20, which showed disperse hybridization on chromosomes of *P. lunatus* and unique signals on chromosomes of *P. vulgaris* (Table 1). On the other hand, one BAC (86I17) that showed a repetitive subtelomeric pattern in *P. vulgaris* gave unique signals in *P. lunatus* (Table 2).

A total of 21 BACs and two bacteriophages showed unique signals on *P. lunatus* chromosomes (Fig. 1d–g). The clones showed no or little repetitive DNA, which was blocked with the C_0t-100 repetitive fraction of *P. vulgaris* DNA when necessary. The relative chromosomal position was established for each individual clone on *P. lunatus* chromosome, which had its identity confirmed by sequential FISH with another clone from the same chromosome

Table 1 List of genetically mapped markers and corresponding BAC clones that showed a repetitive pattern of hybridization on *P. lunatus* chromosomes in comparison to *P. vulgaris* distribution patterns

Linkage group	Marker/gene	BAC clone	*P. lunatus*	*P. vulgaris*[a]
B/B4	*Bng13*	BAC 26B20	Disperse	Unique[b]
	Bng55	BAC 92B6	Disperse	NA
	Bng55	BAC 53G1	Pericentromeric	Pericentromeric
A/B7	*Bng23*	BAC 20F21	Disperse	NA
	Bng28	BAC 12M3	Pericentromeric	Pericentromeric
	Bng47	BAC 267K20	Disperse	Unique
	Bng204	BAC 111O19	Disperse	Disperse
	Bng204	BAC 125P11	Disperse	Disperse
	Phs	BAC 105O5	Disperse	Pericentromeric

NA not analyzed

[a] Determined by Pedrosa-Harand et al. 2009

[b] Blocked with 20 × C_0t-1

on the same slide (Fig. 1f). Multiprobe FISH cocktails containing BAC probes from different chromosomes were hybridized to the same slide to compare relative chromosome lengths. The identification of chromosome 3 was facilitated by the DAPI$^+$ band in the proximal chromosome region after FISH (Fig. 1g).

Nine BACs were mapped on chromosome 3. Three BACs were located on the distal half of the short chromosome arm and six along the long chromosome arm (Fig. 2; Table 2). The centromere was located between BACs 267H4 and 199D13. Eight BACs mapped in *P. lunatus* were present in the cytogenetic map of *P. vulgaris* and the order and position of all BACs were similar in the two species (Fig. 3). BACs 95L13 and 174E13 were selected with the same RFLP (*Bng33*). In this case, the hybridization signals co-localized (Table 2) and the statistical analyses showed the same relative position (Table 3).

Eight markers were mapped on chromosome 4. Three BACs and two bacteriophages were mapped on the short arm and three BACs on the long arm (Fig. 2; Table 2). The markers on the short arm showed no significant difference in position along the chromosome, but for the long arm, different relative positions were observed (Table 3) and all markers conserved the similar order and position on *P. vulgaris* and *P. lunatus* (Fig. 3). BACs 94F8 and 78L24 were not included in the cytogenetic map of *P. vulgaris*; however, these BACs were selected with *Bng160* (Pedrosa-Harand et al. 2009) and mapped at the same position as *Bng151*, which was anchored by BAC 22IJ10 in the *P. vulgaris* cytogenetic map.

The map of chromosome 7 included six BACs, three on the short arm and three on the long arm (Fig. 2; Table 2). All BACs showed different relative positions (Table 3). Five BACs were integrated into the cytogenetic map of chromosome 7 of *P. vulgaris* and complete collinearity was observed (Fig. 3). BAC 86I17 showed a unique signal at the end of the short arm of chromosome 7 of *P. lunatus* (Fig. 2), but was reported as repetitive, showing a subtelomeric pattern, in *P. vulgaris* (Pedrosa-Harand et al. 2009). This BAC was selected with *Bng191* (Pedrosa-Harand et al. 2009), which mapped at the end of linkage group A (Fig. 3) in accordance with the cytogenetic position of this BAC in the *P. lunatus* map.

Table 2 Physical locations of BACs on mitotic metaphase chromosomes from *P. lunatus*

Marker	Clones	C_0t-100	Position[a] ± SD[b]	*n*
Chromosome 3 (C/B3)				
Bng106	BAC 147K17	20×	0.149 ± 0.01	13
Bng12	BAC 142D9	20×	0.172 ± 0.03	11
Bng16	BAC 267H4	20×	0.187 ± 0.02	12
Bng75	BAC 199D13	20×	0.481 ± 0.03	9
Bng114	BAC 116H6	20×	0.562 ± 0.04	8
Bng3	BAC 77J14	50×	0.606 ± 0.03	10
Bng33	BAC 95L13	20×	0.875 ± 0.03	16
	BAC 174E13	20×	0.878 ± 0.02	18
Bng124	BAC 91K16	20×	0.931 ± 0.03	8
Chromosome 4 (B/B4)				
Bng151	BAC 221J10	20×	0.151 ± 0.02	20
	B35		0.171 ± 0.02	11
	B62		0.156 ± 0.02	8
Bng160	BAC 94F8	20×	0.163 ± 0.02	14
	BAC 78L24	50×	0.168 ± 0.01	15
Bng184	BAC 190C15	50×	0.841 ± 0.01	9
Bng103	BAC 162K15	20×	0.850 ± 0.03	9
APA	BAC 86K9	20×	0.910 ± 0.03	9
Chromosome 7 (A/B7)				
Bng191	BAC 86I17	10×	0.092 ± 0.01	20
Bng42	BAC 193F10	20×	0.147 ± 0.02	19
Bng60	BAC 144D16	50×	0.182 ± 0.03	9
Bng204	BAC 122D11	100×	0.749 ± 0.02	18
Bng23	BAC 33M20	40×	0.771 ± 0.02	13
Bng47	BAC 22I21	20×	0.877 ± 0.05	20

[a] Position is relative to chromosome length: *0* telomere of short arm, *1* telomere of the long arm

[b] Standard deviation

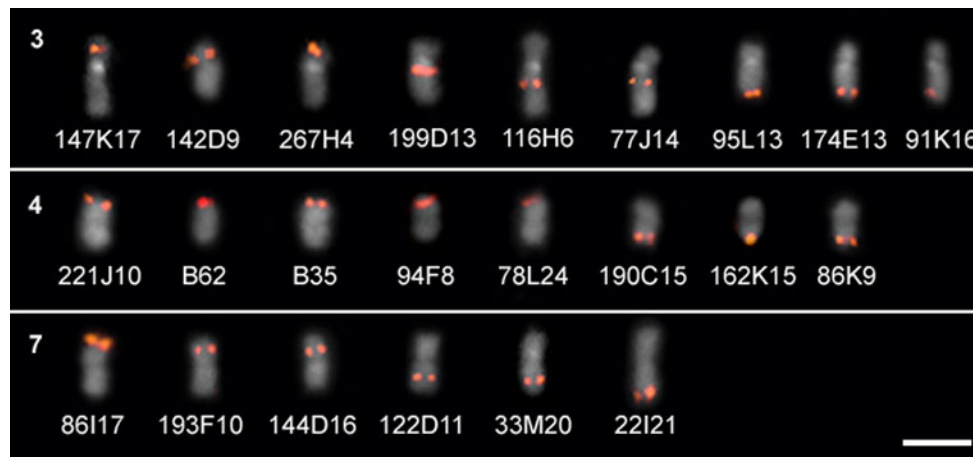

Fig. 2 Localization of clones (*red signals*) on *P. lunatus* mitotic chromosomes. One chromosome for each BAC is shown ordered according to the position of the BAC in the cytogenetic map. The *bar* represents 2.5 µm (color figure online)

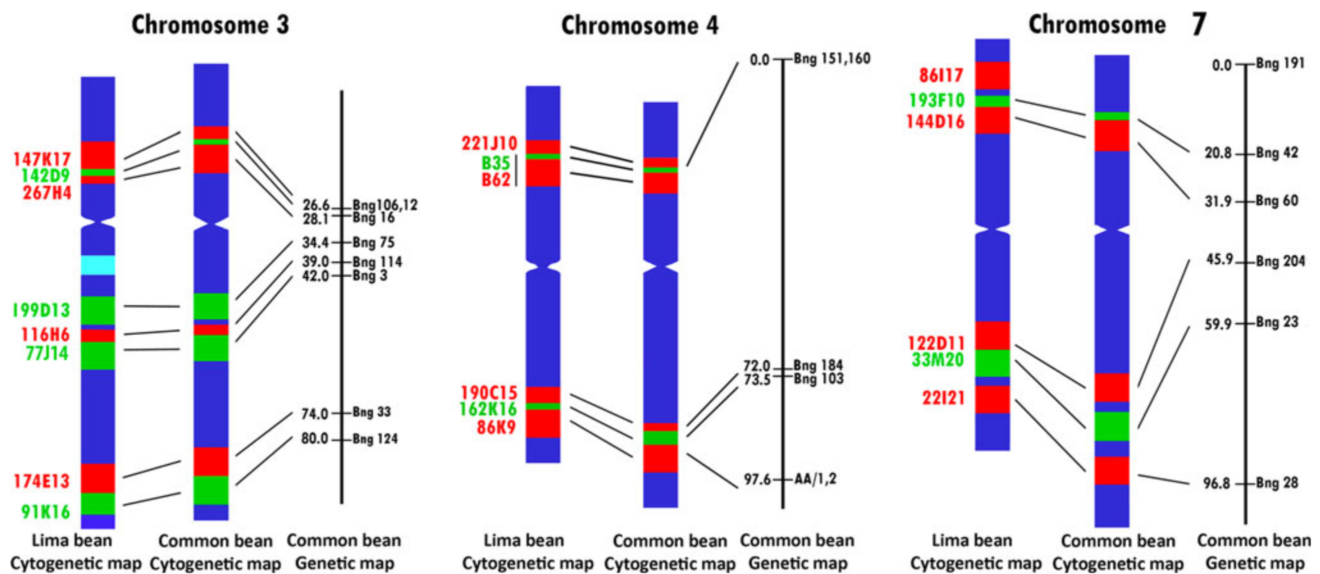

Fig. 3 Comparative cytogenetic maps of chromosomes 3, 4 and 7 between *P. lunatus* and *P. vulgaris*. The common bean cytogenetic map was developed by Pedrosa-Harand et al. (2009) and genetic marker positions of the corresponding *Bng*s in cM are derived from the map of Vallejos et al. (1992). The *green* and *red boxes* represent the positions of different, nearby BAC clones that, in some cases, partially overlap (color figure online)

Discussion

Comparative cytogenetic mapping between *P. vulgaris* and *P. lunatus* was first performed by Bonifácio et al. (2012), who established chromosome homologies and demonstrated conserved synteny between both species. The limited number of markers per chromosome, however, was not enough to confirm collinearity along the chromosomes of these crop species. In the present study, the comparative cytogenetic map of *P. lunatus* chromosomes 3, 4 and 7, using BACs and other genomic clones previously mapped in *P. vulgaris* (Pedrosa-Harand et al. 2009), showed conserved macro-collinearity, or general conservation in the order of genes or single-copy markers along chromosomes, between both species. Most of the markers that were mapped in common bean revealed unique signals on the lima bean chromosomes, confirming this as an efficient and powerful strategy for comparative mapping among *Phaseolus* species.

More than conservation in the order of the markers between the two species, there was a general conservation in the position of the markers along the chromosomes. In the integrated map of *P. vulgaris*, the presence of highly repetitive DNA around extended pericentromeric regions, associated with a suppression of recombination in these regions, was observed (Pedrosa-Harand et al. 2009). Bonifácio et al. (2012) detected similar blocks of heterochromatin around pericentromeric regions in chromosomes

Table 3 Pairwise *T* Student test for the markers mapped in chromosomes of *P. lunatus*

Chromosome 3

Markers	BAC 147K17	BAC 142D9	BAC 267H4	BAC 199D13	BAC 116H6	BAC 77J14	BAC 95L13	BAC 174E13	BAC 91K16
BAC 147K17	–								
BAC 142D9	0.01608	–							
BAC 267H4	0.00025	0.169	–						
BAC 199D13	5.62^{-20}	9.08^{-15}	5.19^{-16}	–					
BAC 116H6	5.39^{-21}	1.17^{-14}	2.87^{-16}	0.00025	–				
BAC 77J14	1.30^{-23}	2.87^{-18}	2.24^{-20}	6.36^{-08}	0.0167	–			
BAC 95L13	4.19^{-34}	1.64^{-28}	6.31^{-31}	1.99^{-20}	2.72^{-16}	1.58^{-17}	–		
BAC 174E13	3.53^{-36}	4.55^{-33}	2.18^{-36}	1.78^{-24}	1.57^{-19}	2.95^{-21}	0.731	–	
BAC 91K16	3.08^{-26}	1.62^{-20}	4.99^{-23}	5.45^{-15}	6.02^{-12}	1.22^{-13}	0.00028	1.77^{-05}	–

Chromosome 4

Markers		B35	B62	BAC 94F8	BAC 78L24	BAC 190C15	BAC 162K15	BAC 86K9
BAC 221J10	–							
B35	0.0124	–						
B62	0.555	0.125	–					
BAC 94F8	0.947	0.3311	0.438	–				
BAC 78L24	0.0049	0.6189	0.0661	0.397	–			
BAC 190C15	6.183^{-36}	1.865^{-25}	5.45^{-22}	4.834^{-29}	3.317^{-35}	–		
BAC 162K15	8.882^{-33}	2.937^{-22}	9.325^{-19}	7.30^{-26}	7.864^{-29}	0.406	–	
BAC 86K9	9.70^{-34}	6.439^{-23}	2.70^{-19}	1.269^{-26}	1.237^{-29}	6.736^{-06}	0.0006	–

Chromosome 7

Markers	BAC 86I17	BAC 193F10	BAC 144D16	BAC 122D11	BAC 33M20	BAC 22I21
BAC 86I17	–					
BAC 193F10	6.68^{-13}	–				
BAC 144D16	1.66^{-12}	0.001	–			
BAC 122D11	1.02^{-49}	2.92^{-43}	1.49^{-36}	–		
BAC 33M20	5.77^{-44}	1.42^{-37}	2.24^{-23}	0.005	–	
BAC 22I21	1.66^{-41}	2.83^{-38}	4.03^{-25}	4.23^{-12}	3.78^{-08}	–

of *P. lunatus*. In this study, pericentromeric heterochromatin detected with few BAC clones was similar to what was observed by Bonifácio et al. (2012). Due to the conservation in the position of markers along the chromosomes in both the species in relation to the pericentromeric heterochromatin, it is possible that suppression of recombination around extended pericentromeric regions is also present in *P. lunatus*.

Evolutionary studies in the genus *Phaseolus* showed *P. vulgaris*, *P. coccineus* and *P. acutifolius* forming one clade (the Vulgaris group), while *P. lunatus* and other species were placed in another group (Delgado-Salinas et al. 2006). Comparative cytogenetic map between *P. vulgaris* and *P. lunatus* suggested three pericentric inversions on chromosome pairs 2, 9 and 10 of *P. lunatus* when compared with *P. vulgaris*, associated with changes in centromere position. For chromosome pairs 1, 5, 6, 8 and 11, no rearrangements were suggested and for chromosome pairs 3, 4 and 7, only one marker was mapped on each chromosome (Bonifácio et al. 2012). However, in the present study, the results showed high conservation of macro-collinearity between chromosomes 3, 4 and 7 of *P. vulgaris* and *P. lunatus*. No indication of inversions, deletions, duplications or translocations was observed, suggesting that chromosome rearrangements are rare between both species. Conservation of synteny has been observed in *Solanum* (Lou et al. 2010), in which 13 BACs were mapped on the chromosome 6 of six species. In this case,

however, despite a general conservation of collinearity, one pericentric and one paracentric inversion differentiated two species from the ancestral chromosome 6. When species with different chromosome numbers from the same genus were compared, however, such as *Daucus carota* ($2n = 18$) and *D. irinitus* ($2n = 22$) and *Cucumis sativus* L. ($2n = 14$) and *C. melo* L. ($2n = 24$), syntenic blocks could be identified but chromosomes were reshuffled by interchromosomal rearrangements (Iovene et al. 2011; Li et al. 2011).

Except for the rare intrachromosomal rearrangements, the main difference observed between *P. vulgaris* and *P. lunatus* chromosomes was due to differential amplification of repetitive DNA. FISH with BAC 86I17 detected subtelomeric heterochromatin in most chromosomes of the common bean (Pedrosa-Harand et al. 2009), but this heterochromatin is apparently absent in lima bean, because this BAC revealed a unique signal on the short arm of chromosome 7. Indeed, *khipu*, a subtelomeric satellite DNA of common bean is present in low copy number in lima bean (David et al. 2009). The opposite situation was observed for BACs 26B20 and 267K20, which showed repetitive DNA that could not be blocked in *P. lunatus*, but gave unique signals in *P. vulgaris*. Indeed, amplification of repetitive DNA at the end of chromosomes of *P. vulgaris* was frequent, as indicated by Pedrosa-Harand et al. (2006), who detected a high number of 45S rDNA loci at the end of chromosomes of several accessions of *P. vulgaris*, while only one 45S rDNA locus was observed in various accessions of *P. lunatus* (Almeida and Pedrosa-Harand 2011). Differential evolution of the repetitive sequences between related genomes was demonstrated, among others, by Zhang et al. (2004) in *Triticum*, in which FISH with various BACs showed genomic-specific repetitive DNA. We concluded that the species *P. vulgaris* and *P. lunatus* have high macro-collinearity for chromosomes 3, 4 and 7 and, except for few inversions (Bonifácio et al. 2012), the main differences were related to differential amplification of repetitive DNA. This information will be useful for lima bean breeders, when exploiting genomic information from common bean.

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