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# Integration of common bean (*Phaseolus vulgaris* L.) linkage and chromosomal maps

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**Abstract** Fluorescent in situ hybridisation of pooled, closely linked RFLP markers was used to integrate the genetic linkage map and the mitotic chromosome map of the common bean. Pooled RFLP probes showed clear and reproducible signals and allowed the assignment of all linkage groups to the chromosomes of two *Phaseolus vulgaris* cultivars, Saxa and Calima. Low extension values for signals originating from clustered RFLPs suggest that these clones are physically close to each other and that clusters in the genetic map are not a result of suppression of recombination due to the occurrence of chromosome rearrangements. For linkage group K, clustering of markers could be associated with proximity to centromeres. High variation in the number of 45S rDNA loci was observed among cultivars, suggesting that these terminal sites are highly recombinogenic in common bean.

**Keywords** Fluorescent in situ hybridisation (FISH) · RFLP markers · Physical mapping · rDNA intraspecific variation · Fabaceae

## Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the major sources of dietary protein in Latin America and Africa (Evans 1986). In recent years, molecular marker analyses have provided new insights into its origin and diversification (Gepts 1998), its process of domestication (Sonnante et al. 1994; Koinange et al. 1996) and the inheritance of disease resistances (Nodari et al. 1993b; Adam-Blondon et al. 1994; Yu et al. 1998; Jung et al. 1999; Vallejos et al. 2000). The first linkage maps of common bean were based on morphological traits (Basset 1991) and isozyme and seed protein markers (Vallejos and Chase 1991) but provided limited genome coverage. A considerable improvement was obtained with the development of three genetic linkage maps with DNA markers (Vallejos et al. 1992; Nodari et al. 1993a; Adam-Blondon et al. 1994). These three established maps have subsequently been integrated, resulting in a core map that has a higher density of markers and an expanded length (Freyre et al. 1998). In spite of these advances, a connection between linkage groups and chromosomes has not been established.

Cytological analysis of the common bean has long been hampered by the small size and overall similarity of its 22 ($n$ = 11) chromosomes. Although different heterochromatin distribution patterns could be discerned after C-banding (Zheng et al. 1991), unambiguous identification of each bean chromosome was not possible. Recently, the identification of most *P. vulgaris* chromosome pairs has been made possible by a combination of double fluorescent in situ hybridisation (FISH) with 45S and 5S rRNA probes, measurements of chromosome sizes, arm ratios and the determination of heterochromatin distribution (Moscone et al. 1999). Furthermore, homeologies to other cultivated beans such as *P. coccineus* (scarlet runner bean), *P. acutifolius* (tepary bean) and *P. lunatus* (lima bean) have been established (Moscone et al. 1999). FISH has also been applied to the giant polytene chromosomes of suspensor cells from developing seeds in *P. vulgaris* and *P. coccineus* to detect some low-copy genes (Frediani et al. 1993; Nenno et al. 1994).

We report here the integration of chromosomal and genetic maps of common bean by means of direct hybridisation of pooled restriction fragment length polymorphism (RFLP) clones from the University of Florida map (Vallejos et al. 1992) to the mitotic chromosomes of two cultivars of the species. Variation in the 45S rDNA loci within the species is also discussed.

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**Table 1** List of RFLP clone pools used per linkage group as probes for FISH. Estimates of total insert size (Vallejos et al. 2001), total coverage in the genetic map (Vallejos et al. 1992) and position on chromosomes of *P. vulgaris* cvs Saxa and Calima are indicated

| Linkage group | RFLP pools | | | Chromosomal localisation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bng clones | Size (kb) | Coverage[a] (cM) | Saxa | | | | Calima | | | |
| | | | | Centre[b] | Extension[c] | n[d] | N[e] | Centre | Extension | n | N |
| A | 40, 168, 170, 191, 199 | 7.4 | 9.2 | 0.11 | 0.07 | 56 | 27 | 0.10 | 0.06 | 37 | 20 |
| B | 13, 55, 103, 184, 224 | 6.0 | 31.7 | 0.84 | 0.08 | 36 | 15 | 0.79 | 0.10 | 65 | 36 |
| C | 3, 29, 114, 123, 155, 164, 175 | 10.2 | 13.3 | 0.67 | 0.09 | 67 | 28 | 0.70 | 0.09 | 48 | 20 |
| D | 82, 89, 90, 98, 105, 115, 117, 148, 174, 178, 180, 212 | 20.6 | 15.9 | 0.38 | 0.12 | 91 | 35 | 0.44 | 0.15 | 58 | 22 |
| E | 49, 65, 152, 166, 205 | 8.0 | 16.8 | 0.20 | 0.10 | 44 | 27 | 0.25 | 0.19 | 64 | 21 |
| F | 22, 31, 38, 43, 58, 73, 91, 213 | 14.2 | 27.7 | 0.88 | 0.06 | 12 | 9 | | | | |
| F′ | 62, 69, 125, 186, 214 | 8.1 | 0 | 0.12 | 0.06 | 44 | 26 | 0.11 | 0.05 | 47 | 29 |
| G | 8, 9, 27, 46, 88, 95, 177, 209, 215 | 16.3 | 17.8 | 0.71 | 0.10 | 52 | 25 | 0.71 | 0.10 | 76 | 33 |
| H | 30, 113, 122, 126, 189 | 7.9 | 0 | 0.90 | 0.06 | 46 | 22 | 0.87 | 0.06 | 34 | 22 |
| I | 100, 121, 172, 218 | 9.0 | 19.2 | 0.09 | 0.08 | 17 | 11 | | | | |
| J | 67, 76, 78, 92, 112, 120, 154, 158, 206 | 15.7 | 0 | 0.07 | 0.05 | 53 | 28 | 0.09 | 0.04 | 22 | 15 |
| K | 79, 93, 109, 129, 132, 149, 163, 226, 231 | 15.5 | 0 | 0.56 | 0.06 | 61 | 35 | 0.56 | 0.05 | 54 | 37 |

[a] Coverage was calculated by adding the genetic distances between markers using the Kosambi addition rule
[b] Central position is a mean value, and it is given as a percentage of chromosome length relative to telomere of short arm
[c] Extension of the signals along the chromosome was calculated using the standard deviation formula
[d] Number of measurements
[e] Number of chromosomes analysed

## Materials and methods

### Plant material

*Phaseolus vulgaris* cv Saxa was obtained from the seed collection of the Botanical Garden of Vienna and is one of the cultivars used by Moscone et al. (1999) for constructing the idiogram of the species. *P. vulgaris* cv Calima and XR-235-1-1 are breeding lines used by Vallejos et al. (1992) to establish a common bean linkage map.

### Chromosome preparation

Root tips obtained from germinating seeds were pre-treated in 2 m*M* 8-hydroxyquinoline for 4–5 h at 16 °C, fixed in methanol/acetic acid 3:1 (v/v) and stored in fixative at –20 °C up to several weeks. For digestion of the cell wall, root tips were macerated in 3% (w/v) cellulase Onuzuka R-10 (Serva, Heidelberg) plus 30% (v/v) pectinase (Sigma-Aldrich) in 0.01 *M* citric acid-sodium citrate buffer, pH 4.8, for 1 h at 37 °C. Digested material was transferred to a drop of 45% acetic acid and flamed before squashing.

### DNA Probes

R2, a 6.5-kb fragment of an 18S-5.8S-25S rDNA repeat unit from *Arabidopsis thaliana* (Wanzenböck et al. 1997), and D2, a 5S rRNA clone from *Lotus japonicus* (Pedrosa et al. 2002), were used for chromosome identification. A sub-set of the genetically mapped RFLP markers, designated as Bng clones (Vallejos et al. 1992), which are derived from a *Pst*I genomic library (Chase et al. 1991), were used for correlation to the University of Florida genetic map. Except for linkage group F, one pool of RFLP clones was selected per linkage group, based on their close position in the genetic map (Table 1).

Clones were amplified by conventional DNA mini-preparation technique or by the polymerase chain reaction (PCR). The PCR primers 5′-GCCAAGCTTGCATGCCTG-3′ and 5′-TCCTCTAG-AGTCGACCTG-3′ were designed to amplify whole inserts. The thermocycler was programmed as follows: 25 cycles of 50 s at 95 °C, 50 s at 52 °C and 2 min 30 s at 72 °C and a final extension of 10 min at 72 °C. Insert sizes were obtained from Vallejos et al. (2001). Clones of the same linkage group region were pooled together before labelling. All probes were labelled by nick translation (Roche Diagnostics, Life Technologies) with Cy3-dUTP (Amersham Pharmacia Biotech), except for R2, which was labelled with biotin-14-dATP (Life Technologies).

### Fluorescence in situ hybridisation (FISH)

Slides were selected and pre-treated as described in Pedrosa et al. (2001). Chromosome and probe denaturation, post-hybridisation washes and detection were performed according to Heslop-Harrison et al. (1991), with modifications described in Pedrosa et al. (2002) for hybridisation without blocking DNA. Biotin-labelled probes were detected using ExtrAvidin-FITC conjugate (Sigma-Aldrich) in 1% (w/v) bovine serum albumin. All preparations were counter-stained and mounted with 2 µg/ml DAPI in Vectashield (Vector). Re-probing of the slides for localisation of different DNA sequences on the same cell was performed according to Heslop-Harrison et al. (1992).

### Image analysis

Photographs were taken on a Zeiss Axioplan (Carl Zeiss) equipped with a mono-cool-view CCD camera (Photometrics, Tucson). Images from the camera were combined and pseudo-coloured using the IPLab spectrum software (IPLab, Fairfax). Measurements of arm and chromosome lengths as well as the distance of FISH signals to the closest telomere were performed using the ‘analyse – measure length’ function of the same software. Up to four signals were measured per homologue. Digital images were imported into ADOBE PHOTOSHOP version 5.5 for final processing.

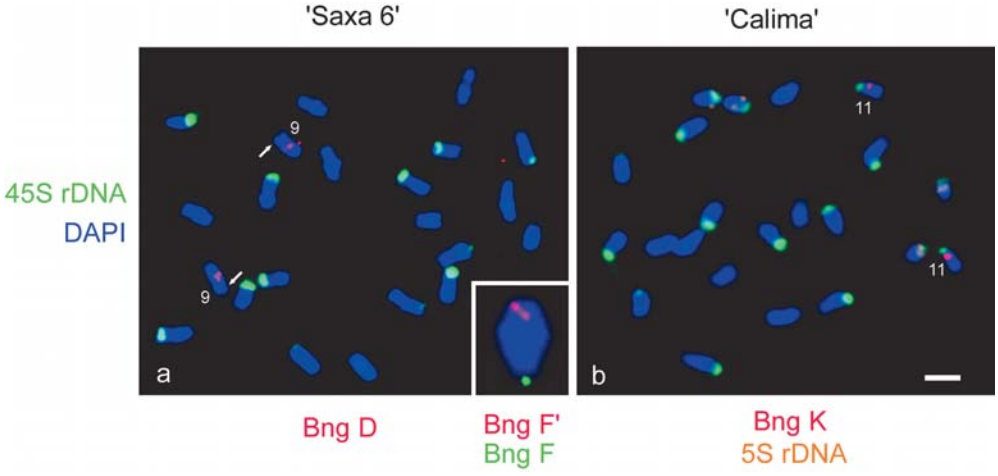


**Fig. 1** Localisation of RFLP pools on chromosomes of *Phaseolus vulgaris* cv Saxa (**a**) and cv Calima (**b**) by FISH. RFLP pools D, F′ and K are seen in *red* and RFLP pool F is seen in *green* (*insert* in **a**). 45S (*green*, **a**, main picture, and **b**) and 5S rDNA probes (*orange*, **b**) were used for chromosome identification. In each picture, relevant chromosomes were *numbered*. *Arrows* in **a** indicate centromere position. Brighter DAPI staining of centromeric heterochromatin is clearly visible in this example. *Bar* (**b**): 2.5 μm

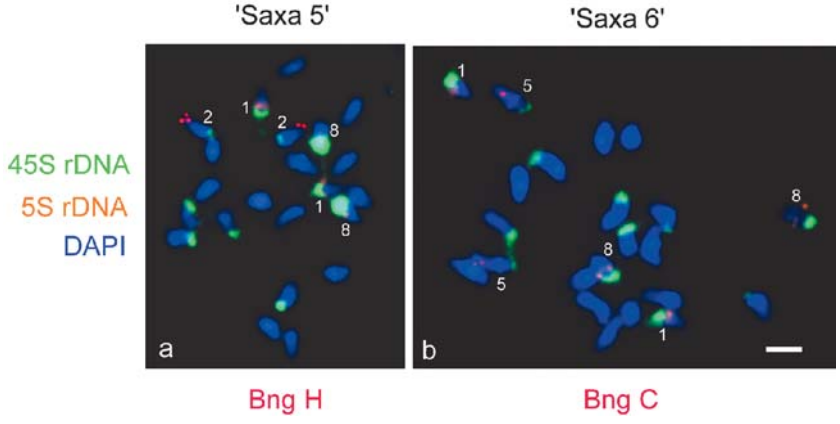


**Fig. 2** Localisation of RFLP clones on chromosomes of *P. vulgaris* cytotypes Saxa 5 (**a**) and Saxa 6 (**b**) RFLP pools H (**a**) and C (**b**) are seen in *red*, 45S rDNA in *green* and 5S rDNA in *orange*. Relevant chromosomes are indicated by *numbers*. Note the presence of ten 45S rDNA loci in **a** and 12 loci in **b**. *Bar* (**b**): 2.5 μm

### Construction of idiograms

Ten well-spread complete metaphases were analysed for each cultivar. Relative length and arm ratio (long arm/short arm) were calculated for each chromosome. Additionally, size and position of marker clusters (45S and 5S rDNA) were measured. Pairs were established according to chromosome morphology and location of marker probes and mean values were calculated for each pair. For *P. vulgaris* cv Saxa, two idiograms were constructed, due to variation among individuals. The position of the pooled RFLP clones on the chromosome was calculated as a percentage of the chromosome length, with mean values representing its central location. The extension of the pool of clones along the chromosome was calculated using the standard deviation formula. Identification of labelled chromosomes was based on chromosome morphology, presence/position of marker clones and calculation of relative size of the labelled chromosome pair in relation to the other chromosomes of the cell. Chromosomes were numbered according to Moscone et al. (1999). The Mann-Whitney nonparametric test of variance and the calculation of Pearson's (r) and Spearman's ($r_s$) correlation coefficients were performed using SPSS RELEASE 7.0 (SPSS Inc., Chicago, IL).

## Results

### Physical localisation of RFLP clones

The Bng clones were originally selected to range between 0.5 kb and 2.5 kb in size (Chase et al. 1991). It has been reported that individual probes in this size range are difficult to detect by FISH in plant chromosomes (Jiang and Gill 1996). To increase the sensitivity of detection, we used as FISH probes pools of RFLP clones that have been previously mapped close to each other (Vallejos et al. 1992). Clusters of markers, as present in linkage groups F, H, J and K, were preferentially used (Table 1). Selected pools of fluorescently labelled RFLP clones were hybridised to mitotic metaphase chromosomes because at this stage chromosomes are most condensed, and the signal from probes localised not far from each other are expected to coalesce and yield a stronger signal. Results obtained in these experiments were easily integrated with the most detailed idiogram of the species. This idiogram has been established previously using mitotic metaphase chromosomes (Moscone et al. 1999).

With few exceptions, the clone pooling approach generated clear and highly reproducible signals on homologous regions of a chromosome pair (Figs. 1–3). This was achieved by pooling a minimum of five RFLP clones, comprising a total insert size between 6 kb and 8 kb, as exemplified by pools A, B, E, F′ and H (Table 1). Pools F and I, however, produced relatively weak signals and could only be detected in a subset of cells. Intensity of signals could not be directly correlated to total insert size or to proximity of the clones in the genetic map. These

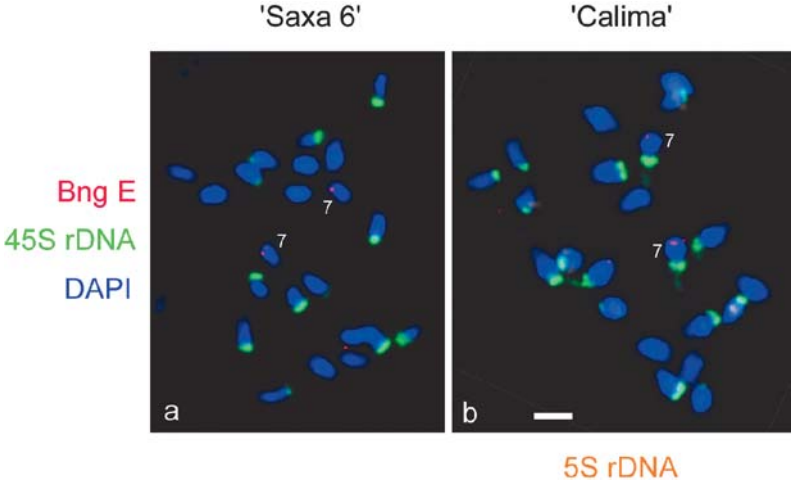


**Fig. 3** Mapping of RFLP pool Bng E to chromosome 7 of *P. vulgaris* cv Saxa (**a**) and cv Calima (**b**) Bng E is seen in *red*, 45S rDNA in *green* and 5S rDNA in *orange* (**b**) Note the presence of 12 45S rDNA loci in **a** and 16 loci in **b** *Bar* (**b**): 2.5 μm

results suggested that the physical distance between marker loci represented in the pool, or a higher order of chromatin organisation, may affect the signal intensity generated by a pool of probes. Although all probes were similarly labelled, we cannot exclude variation in labelling efficiency among different reactions as another factor affecting signal intensity. Depending on the pool used, signals were visualised as a few dots scattered along a chromosome region (e.g. pool D, Fig. 1a) or as a single, compact band (e.g. pool K, Fig. 1b). For a few probes, additional random background signals were occasionally visible on other chromosome regions.

## Mapping of RFLP clones on *P. vulgaris* Saxa chromosomes

Each of the 11 linkage groups was assigned to a distinct *P. vulgaris* Saxa chromosome. The chromosome pair bearing a signal was identified by its morphology and the presence and position of rDNA loci. In the cases where chromosomes had a similar morphology, the two pools of probes were hybridised to the same preparation. In all of these experiments, each pool was detected on different chromosome pairs. In general, there was no correlation between chromosome and linkage group sizes. The relative position of each RFLP clone pool along the chromosome is listed in Table 1.

The idiogram we obtained for cv Saxa was very similar to the idiogram reported earlier by Moscone et al. (1999) for the same cultivar. However, while Moscone et al. (1999) classified chromosome 9 as metacentric, we determined it to be subtelocentric (see arrows in Fig. 1a).

This discrepancy can be explained by the fact that the centromere position of chromosome 9 is particularly difficult to determine due to the absence of a conspicuous DAPI heterochromatic band.

Linkage group F was originally represented by two regions of linked markers with a 35.9-cM (LOD: 2.22) gap in between (Vallejos et al. 1992). The integrity of this linkage group has been recently confirmed by the incorporation of additional marker loci in the gap region (Vallejos et al. 2001) and by the FISH data presented here. When metaphase spreads were probed with the F and F′ pools, each from one end of the linkage group, both pools hybridised to the sub-terminal regions of the same chromosome (Figs. 1a, 4). This suggests that the original gap corresponded to a large proportion of the central region of this chromosome.

Except for group F, the orientation of linkage groups on the corresponding chromosomes could not be unambiguously established because only one pool of clones per linkage group was used for FISH analysis. Nevertheless, we propose that, at least for linkage groups A, F, I and J, the marker order should be reversed, based on the use of telomeric-subtelomeric probes. This orientation would keep with the convention of presenting chromosomes/linkage groups with the short arm on top and the long arm at the bottom. It could be argued that an entire linkage group of the common bean could correspond to a very small stretch of chromosome. The kb/cM relationship is known to vary along chromosomes, and extremely high ratios have been reported for grasses with large genomes and high repetitive DNA content (Gill and Friebe 1998). However, extremely high kb/cM ratios have not been detected in plants with small genomes such as Arabidopsis or rice (Schmidt et al. 1995; Cheng et al. 2001b). *P. vulgaris* has a small genome, and it is therefore expected to be similar.

## Polymorphisms in cv Saxa

We detected polymorphisms for the size and number of 45S rDNA loci among individuals of cv Saxa. Five individuals had five 45S rDNA loci in the haploid genome (Fig. 2a), as reported by Moscone et al. (1999), but six loci were detected in 11 individuals. The additional locus was identified on the short arm of chromosome 5 (Fig. 2b). In addition, the sizes of the 45S rDNA clusters on chromosomes 8 and 11 were reduced and increased ($P = 0.000$), respectively, in the latter individuals. Due to these differences, we defined these two groups of individuals as cytotypes Saxa 5 – for individuals carrying

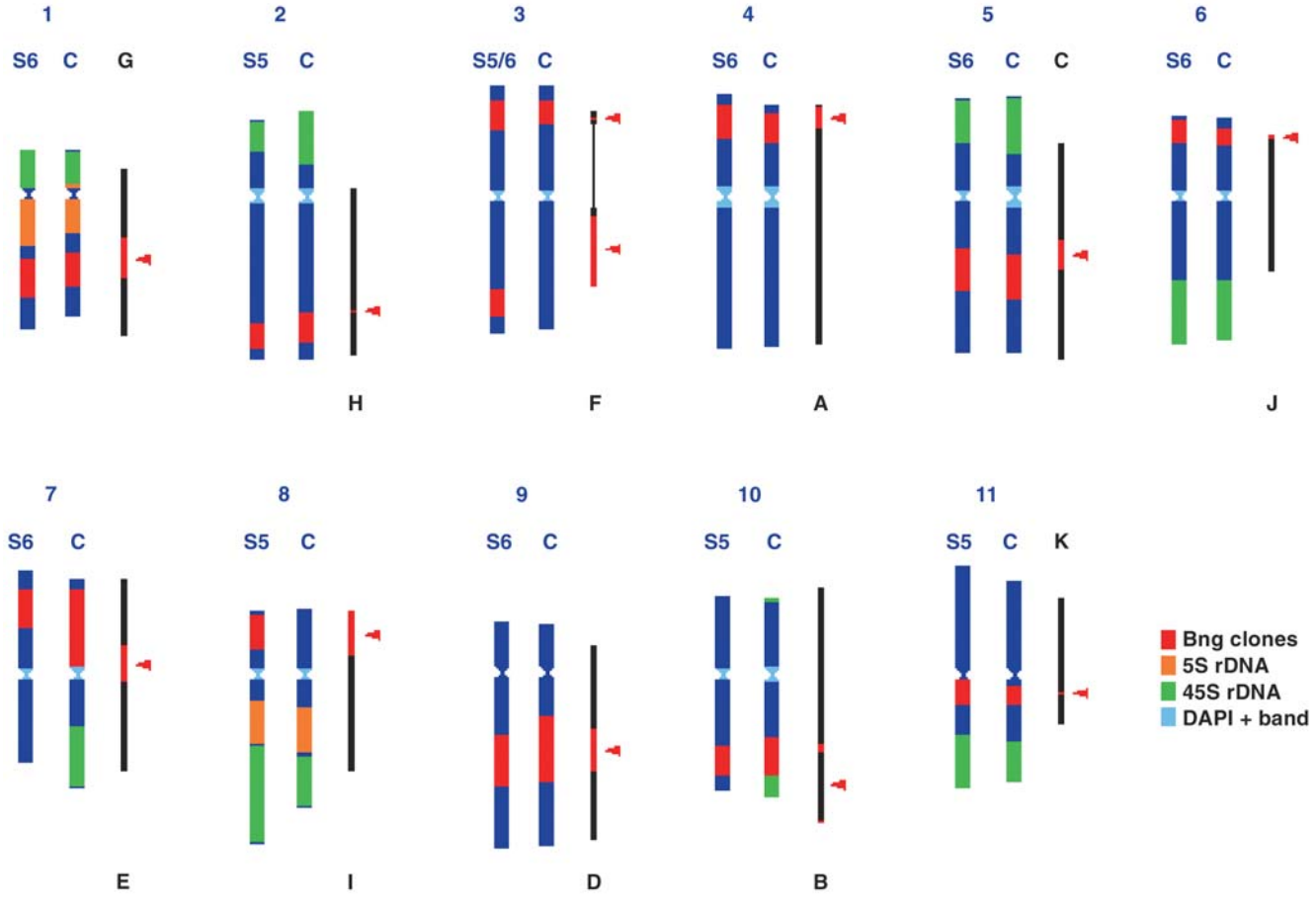


**Fig. 4** Idiogram of *P. vulgaris* cv Saxa (*S*) and cv Calima (*C*) in comparison to the University of Florida genetic map (Vallejos et al. 1992). For cv Saxa, *S 5* and *S 6* represent the respective cytotypes in which the measurements for the position of each RFLP pool were done. Position of RFLP pools are indicated in *red* in both chromosomes and linkage groups, and marked by *arrowheads*. Numbering of chromosomes is according to Moscone et al. (1999). For linkage group F, two RFLP pools were used (F′ on top, measured from cytotype Saxa 5; and F on *bottom*, measured from cytotype Saxa 6; position of F′ was confirmed by double hybridisation, see Fig. 1a). Designation of linkage groups is written at the bottom when orientation has been reverted in comparison to original map

five sites of 45S rDNA – and Saxa 6 – for individuals carrying six sites. Idiograms for both cytotypes were calculated and differences are presented in Fig. 5.

## Mapping of RFLP clones in cv Calima

The variation in number of 45S rDNA loci detected among individuals of *P. vulgaris* Saxa prompted us to investigate the complement of the two cultivars used for establishing the University of Florida genetic map: Calima and XR-235-1-1. 45S rDNA loci were observed on four chromosome pairs of cv XR-235-1-1 (data not shown), whereas in Calima 45S rDNA sites were detected on eight chromosome pairs (Figs. 1b, 3b).

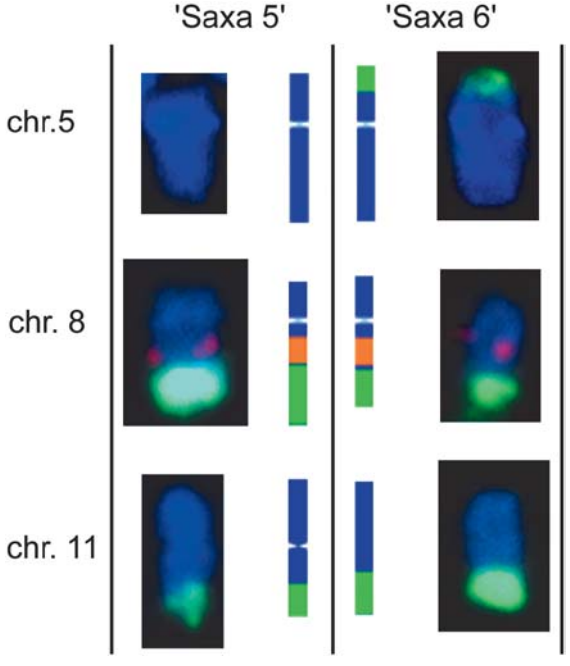


**Fig. 5** Variation in the size of the 45S rDNA clusters from chromosomes 5, 8 and 11 between cytotypes Saxa 5 and Saxa 6 of *P. vulgaris*. 45S rDNA probe is visualised in *green* and 5S rDNA probe in *red*. Idiograms represent mean values from five cells per cytotype. The position of the centromere of chromosome 11 from cytotype Saxa 6 was not determined

**Table 2** Comparison between linkage group and chromosome sizes in common bean. Linkage group values were obtained from Vallejos et al. (1992). Chromosome values were obtained from measurements in *Phaseolus vulgaris* cv Calima

| Chromosome number[a] | Linkage group name | Chromosome size[b] (%) | Chromosome arm ratio[c] | 45S rDNA size[d] | 5S rDNA size | Chromosome size[e] (Mbp) | Number of RFLPs[f] | Linkage group length[g] | Mbp/cM |
|---|---|---|---|---|---|---|---|---|---|
| 1 | G | 6.91 | 2.74 | 20 | 30 | 44 | 20 | 73.6 | 0.60 |
| 2 | H | 10.33 | 1.94 | 21 | | 66 | 20 | 73.3 | 0.90 |
| 3 | F | 10.08 | 1.22 | | | 64 | 22 | 78.0 | 0.82 |
| 4 | A | 10.06 | 1.65 | | | 64 | 25 | 104.7 | 0.61 |
| 5 | C | 10.59 | 1.58 | 22 | | 67 | 24 | 94.6 | 0.71 |
| 6 | J | 9.26 | 1.87 | 27 | | 59 | 20 | 60.7 | 0.97 |
| 7 | E | 8.65 | 1.20 | 29 | | 55 | 8 | 85.5 | 0.64 |
| 8 | I | 8.24 | 2.01 | 25 | 20 | 52 | 7 | 70.6 | 0.74 |
| 9 | D | 9.23 | 3.68 | | | 59 | 28 | 85.8 | 0.69 |
| 10 | B | 8.28 | 1.57 | 13 | | 53 | 8 | 104.0 | 0.51 |
| 11 | K | 8.37 | 1.16 | 21 | | 53 | 42 | 56.2 | 0.95 |
| Total | | 100.00 | | | | 637 | 224 | 887.0 | 0.72 |

[a] Chromosome numbers are according to Moscone et al. (1999)
[b] Relative chromosome length is given as a percentage of the total complement length
[c] Arm ratio = long arm/short arm
[d] Sizes of rDNA loci were measured from FISH signals and are indicated as percentage of chromosome length. Note that, using this method, values are probably overestimated.
[e] Chromosome sizes in Mbp were calculated based on the relative chromosome lengths and a total genome size of 637 Mbp (Arumuganathan and Earle 1991)
[f] Number of RFLP loci per linkage group
[g] Lengths of linkage groups are given in centiMorgans

Mapping the same pools of RFLP clones in Calima showed that the positions of the pools were, with few exceptions (see below), identical in Calima and Saxa (Fig. 4). Similarly, except for the pool from linkage group E, the fluorescent signal from the hybridizing probes extended over comparable lengths (Table 1). A few clear differences were observed between the Calima and Saxa idiograms. These differences related to the number, size and position of the 45S rDNA clusters. In contrast to the five or six 45S rDNA loci detected in Saxa, Calima displayed nine loci. Extra sites were detected on chromosomes 7L (see Fig. 3), 10S (a minor locus) and 10L (Fig. 4). The 45S rDNA locus of chromosome 7 of Calima was in a *decondensed* state in the majority of the cells analysed. This indicates that this locus is transcriptionally active (Fig. 3b).

The use of previously mapped single-copy sequences as FISH probes has allowed us to assign each linkage group to a bean chromosome. Chromosomes were numbered according to descending size in cv Tschermak's fadenlose Wachs – Wax, with the largest named 1 and shortest 11 (Moscone et al. 1999). In Table 2 we present the relative chromosome lengths, arm ratios and rDNA distribution of each chromosome of cv Calima. Their DNA content (in Mbp) was estimated according to its relative length and the estimated size of the *P. vulgaris* genome. Correlation to linkage groups of the University of Florida genetic map (Vallejos et al. 1992) is also presented. Because this map has been integrated to the other genetic maps of the species (Freyre et al. 1998; Vallejos et al. 2001), chromosome numbers can be used to unify the linkage group nomenclature of common bean.

### Comparison of physical and genetic distances

Hybridisation pools were made up of 5 to 12 clones of marker loci. In some cases, pool members represented loci that have been mapped in a cluster (0 cM), while in other cases they have been mapped over a map distance of up to 31 cM. The extension of the FISH signal from each of the pools was calculated and correlated to the map distance covered by the marker loci represented in the pool (Table 1). A significant ($P = 0.01$) correlation ($r_s = 0.857$) between these two parameters was detected. These results suggested that clustering of clones represented physical proximity among them, and was not the result of suppressed recombination.

As in Saxa, no correlation was observed between linkage group and chromosome sizes ($r = 0.28$) or between the number of markers per linkage group and chromosome size ($r = 0.21$) in Calima (see Table 2). The presence of large rDNA clusters may contribute to the relatively small size of some linkage groups such as C and I. However, it does not seem to be the only reason for the lack of correlation (see Fig. 4).

## Discussion

The use of pools of linked RFLP markers as FISH probes on metaphase chromosomes proved to be a very reliable approach to establish the association between molecular marker-based linkage groups and individual *P. vulgaris* chromosomes. Many of the efforts to correlate genetic and chromosome maps by FISH in plants have been based on multigene families organised in clusters (Pedersen et al. 1995; Fuchs et al. 1998). Although these loci can be reproducibly detected via FISH with

relatively small probes, they have the disadvantage of not being available in large enough numbers to cover an entire genome. Large insert genomic clones, such as BACs (Dong et al. 2000; Kulikova et al. 2001; Pedrosa et al. 2002), have also been used for the same purpose, but this approach is more demanding. A BAC library has to be screened with previously mapped clones first, the correspondence between marker and clone has to be confirmed and then the selected BAC clone is used as a FISH probe, provided it doesn't have a very high proportion of repetitive DNA (Jiang and Gill 1996). The use of pooled RFLP markers as FISH probes for mitotic metaphase chromosomes appears to be an efficient method to determine the chromosomal identity of established molecular marker-based linkage groups.

Two loci for the 5S rDNA repeat were detected on chromosomes 1 and 8 of the species. Based on the uniformity of cluster number and size for the four cultivars we and Moscone et al. (1999) analysed, we can conclude that the 5S rDNA loci of *P. vulgaris* are conserved. In contrast, a high degree of variation in size and number of loci was observed for the 45S rDNA sequence within and between cultivars, although in all cases individuals were structurally homozygous. The Mesoamerican lines Jamapa (A. Pedrosa, unpublished results) and XR-235-1-1 displayed three and four loci, respectively, whereas the Andean cultivars Tschermak's fadenlose Wachs – Wax, Saxa and Calima had five to eight chromosome pairs bearing 45S rDNA loci (Moscone et al. 1999 and present work). Analysis of this small sample seems to indicate that a higher degree of amplification and dispersion of 45S rDNA units has occurred among Andean cultivars.

Variation in the size of the signal in a locus was interpreted as a difference in copy number between alleles. Intraspecific variation for the 45S rDNA copy number has been documented for a variety of organisms including plants (Rogers and Bendich 1987a, b). However, only limited variation in number and/or position of 45S rDNA or 5S rDNA loci has been reported for plants (Fukui et al. 1994; Fuchs et al. 1998; Cerbah et al. 1999). In *Allium,* it has been proposed that distal 45S rDNA sites become mobile by frequent recombination and/or conversion events to overcome replication-mediated shortening at the chromosome ends when this sequence acts as telomeres (Pich et al. 1996). A similar reason for increased recombination in rDNA loci can not apply to *P. vulgaris*, since the Arabidopsis-like telomeric repeat was detected at all chromosome ends in Saxa, also distally to rDNA sites (A. Pedrosa, unpublished data).

In spite of the presence of multiple loci, no intraspecific variation in 45S rDNA ITS sequence was detected by Delgado-Salinas et al. (1999), indicating that the 45S DNA loci of *P. vulgaris* have undergone rDNA homogenisation. Solid evidence in support of recombination among sub-telomeric 45S rDNA loci has been presented for man and apes (Arnheim et al. 1980) and for tetraploid cottons (Wendel et al. 1995). Thus, the sub-telomeric position of the 45S rDNA loci in *P. vulgaris* suggests that rDNA homogenisation in this species occurs predominantly via recombination of non-homologous rDNA loci. This may explain the allelic differences in copy number and loci number in common bean.

We have previously observed the presence of inversions in chromosome regions corresponding to clustered markers in the genetic map (Pedrosa et al. 2002). The detection of clustered markers dispersed on relatively small chromosome regions in common bean, however, suggested that no major chromosome rearrangement is present within the species. This is consistent with the fact that genetic maps constructed from different intraspecific crosses revealed the same order of markers with minor exceptions (Freyre et al. 1998; Jung et al. 1999). Clusters of markers, observed in the University of Florida map, may represent regions with higher levels of polymorphism between progenitors or a bias in the sampling procedure (Vallejos et al. 1992). Only in the case of linkage group K, suppression of recombination may be associated with proximity to the centromere.

The high-density linkage maps of tomato and rice showed significant correlation between chromosome and linkage group sizes (Tanksley et al. 1992; Harushima et al. 1998; Cheng et al. 2001a). A similar comparison in common bean revealed no correlation when chromosomes were compared to the University of Florida map and to the core map of the species ($r = 0.34$), although the latter map has reached the total length predicted from linkage analysis (Vallejos et al. 1992; Freyre et al. 1998). Different reasons could account for this lack of correlation in common bean, such as different rates of recombination among chromosomes, presence of undetected chromosome rearrangements and incompleteness of the genetic maps. To address these questions, a more detailed comparison of Mbp/cM ratios along chromosomes would be required. Fine physical mapping using single-copy, large-insert probes and more distended chromosomes will be performed to further improve the comparison of genetic and physical distances in common bean.

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