

Chromosome maps of legumes

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Abstract

Legumes are of immense importance as food and feed, and for sustainable agriculture, due to their ability to fix nitrogen. Here, the chromosome maps of the legumes soybean (*Glycine max*), *Lotus* (*Lotus japonicus*), and red clover (*Trifolium pratense*) are reviewed. These species have relatively small chromosomes and therefore are difficult to exploit for chromosome studies. Nevertheless, the identification of individual chromosomes became feasible, and chromosome maps have been developed applying image analysis and fluorescence *in-situ* hybridization. For *Lotus japonicus*, e.g. detailed chromosome maps have been developed using the information of genetic linkage maps. Future prospects of further legume chromosome mapping for breeding and genetic purposes are discussed.

Introduction

There are 18 000 species of legumes that comprise the third-largest family of flowering plants and display a striking variety of plant types ranging from small annual herbs to massive tropical trees. Among the legumes the subfamily Papilionoideae contains the majority of pulse crops such as pea (*Pisum sativum*, $2n=14$, 5000 Mb), alfalfa (*Medicago sativa*, $2n=16$, 1600 Mb), and soybean (*Glycine max* (L.) Merr., $2n=40$, 1100 Mb). Legumes cover 180 000 000 ha and 12–15% of the arable land in the world (Graham & Vance 2003); in addition to their agronomic importance as food for humans and livestock, legumes are one of the major nitrogen fixation systems in the biosphere due to their unique ability to establish a symbiotic relationship with several genera of bacteria such as rhizobia (Stacey *et al.* 2006).

For legume species with large chromosomes, such as *Vicia faba* ($2n=12$) and *Pisum sativum* ($2n=14$), it is possible to use ordinary karyotyping and/or banding methods for chromosome identification. A comprehensive survey of the molecular and cytological features of the chromosome complement was provided for *V. faba* based on FISH and various Giemsa and fluorescence banding patterns (Fuchs *et al.* 1998a). Physical mapping of DNA sequences by FISH plays an important role connecting information from linkage and chromosome maps, as has been demonstrated for pea (Fuchs *et al.* 1998b). In the case of legumes with small chromosomes, identification of individual chromosomes and their centromeric positions is difficult, especially after condensating pretreatment with colchicine, 8-hydroxyquinoline or cold water. The chromosome image analyzing system (CHIAS) for small chromosomes makes use of distinct stainability

along mitotic prometaphase chromosomes, due to uneven condensation, a feature specific to small plant chromosomes (Fukui & Iijima 1991). The density profiles at the center line of both chromatids (mid-rib line) of prometaphase chromosomes allowed establishment of the first chromosome maps of several legumes with small chromosomes (Yanagisawa *et al.* 1991, Ito *et al.* 2001, Sato *et al.* 2005).

Here we focus on attempts to integrate genetic, physical and chromosome density mapping into comprehensive chromosome maps for soybean (*G. max*), *Lotus* (*Lotus japonicus*) and red clover (*Trifolium pratense*) as representative legumes with small chromosomes.

Development of a preliminary chromosome density-based map of *G. max*

Soybean is an economically important legume with 40 chromosomes of approximately 17 μm length during mitotic metaphase (Yanagisawa *et al.* 1991). Figure 1A and B show *G. max* chromosomes at metaphase and prometaphase, respectively. Although typical condensation patterns (CP) appeared on the prometaphase chromosomes, their similarity prevented a complete identification of all chromosomes. One pair of the chromosomes shows a high arm ratio of 5.7; that of the other chromosomes ranges from 1.3 to 2.5. Based on CP, the *G. max* chromosomes were classified into five groups (A to E, Figure 1C). Group A is represented by the chromosome pair with the arm ratio of 5.7 and a nucleolus organizing region (NOR) close to the end of its short arm. Such a chromosome pair occurs in all nine species of the genus *Glycine* tested so far (Yanagisawa *et al.* 1991) and is therefore considered as 'marker chromosome' within the genus *Glycine*. The small satellite distal to the NOR is not always visible. Group B chromosomes display three condensed regions: one on the short arm and two on the long arm. Group C includes 10 chromosomes with an arm ratio greater than 1.6 and one condensed region on either side of the centromere. These submetacentric chromosomes are divided into two subgroups. Eight chromosomes have the larger condensed region on the long arm and two have it on the short arm. Group E comprises 12 nearly metacentric chromosomes with arm ratios less than 1.2 and one condensed region on either side of the centromere. Eight chromosomes have condensed regions of different size and four of equal size. Group D includes 14

chromosomes with arm ratios between that of group C and E, respectively, and with a highly variable CP.

Previous FISH studies revealed a single 18S-5.8S-28S rDNA locus (Skorupska *et al.* 1989) and a single 5S rDNA locus (Shi *et al.* 1996) for *G. max*. Figure 1D shows the same for the wild annual progenitor *G. soja*. One 45S rDNA locus was detected in 17 accessions of 14 diploid species of the genus *Glycine* including *G. max* and *G. soja* (Singh *et al.* 2001). FISH on pachytene chromosomes allowed assignment of seven gene-rich BAC of linkage group L collinearly to the long arm of the acrocentric chromosome 19, as well as detection of ancient duplications within the soybean genome (Walling *et al.* 2006).

Integrated maps for *Lotus japonicus* and *Medicago truncatula* chromosomes

Genomics has progressed rapidly in recent years for *L. japonicus* (Regel) Larsen ($2n = 12$, 472.1 Mb) and *M. truncatula* L. ($2n = 14$, 500 Mb) (VandenBosch & Stacey 2003; Young *et al.* 2005). *L. japonicus* is an autogamous diploid and a perennial temperate pasture species of a short life cycle (2–3 months). Condensation and FISH analyses were performed on mitotic prometaphase chromosomes (Ito *et al.* 2000; Pedrosa *et al.* 2002). Prometaphase chromosomes and the corresponding idiograms for two accessions, Miyakojima and Gifu, are shown in Figure 2. The idiograms are based on CHIAS3 (<http://www2.kobe-u.ac.jp/~ohmido/cl/chiasIII/index.htm>) analysis of three Miyakojima and seven Gifu prometaphase plates selected according to similar chromosome lengths. Both accessions have six chromosome pairs with distinct condensation patterns after Giemsa staining. Faint, unstable and small condensed (FUSC) regions were observed at some chromosome termini of accession Miyakojima (Figure 2A, arrows) but not of accession Gifu. The nuclear DNA content of both accessions was measured by flow cytometry and estimated to be 472.1 ± 3.98 Mb for Miyakojima and 442.8 ± 2.32 Mb for Gifu. The FUSC regions on the chromosomes of Miyakojima might contribute to its larger genome size.

The 45S rDNA loci were on chromosome 2, 5, and 6 (Ito *et al.* 2000) and the 5S rDNA locus was mapped on the short arm of chromosome 2 (Figure 2E).

The chromosome maps based on the CP were combined with the *Lotus* linkage maps (Sato & Tabata

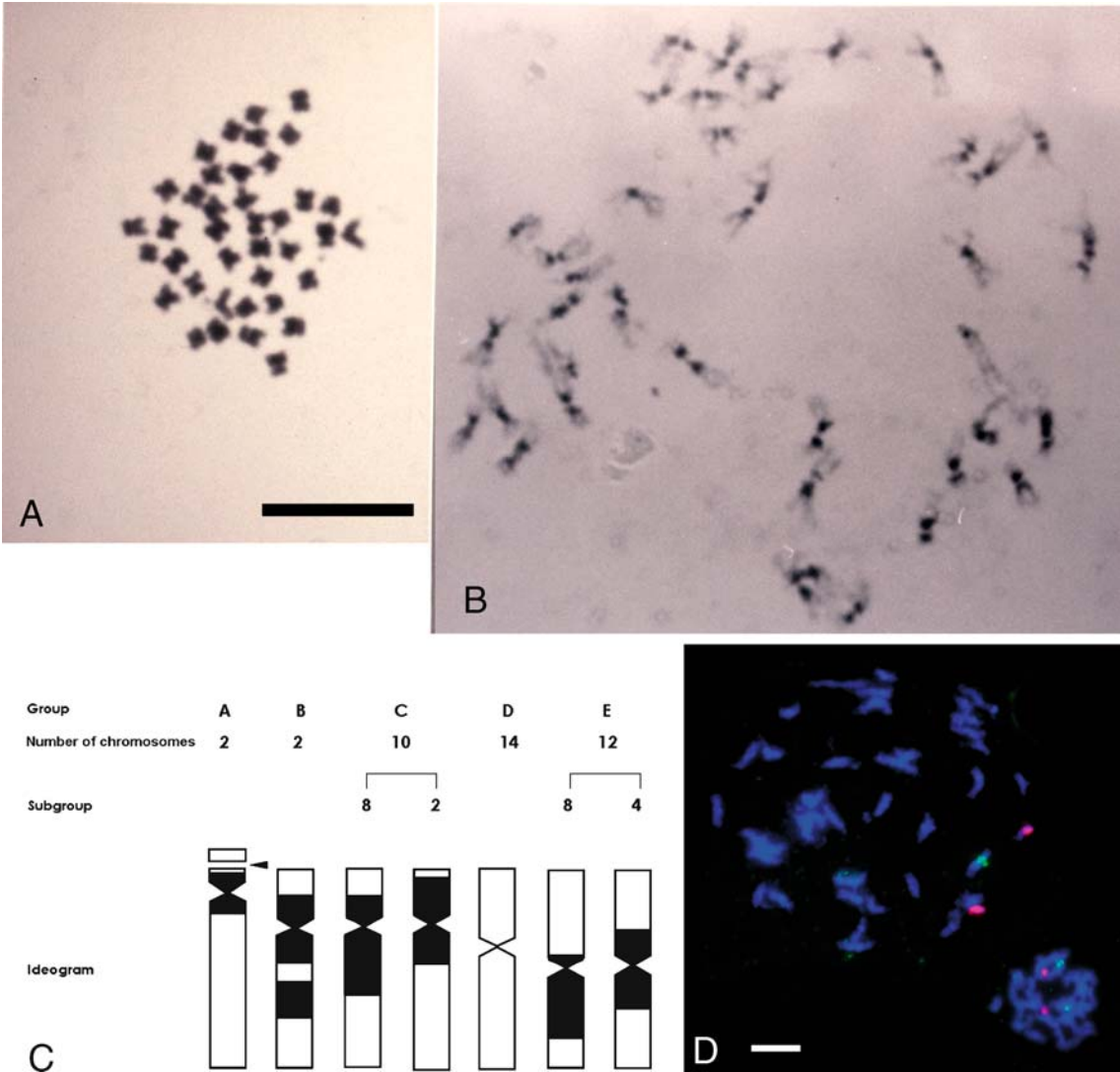
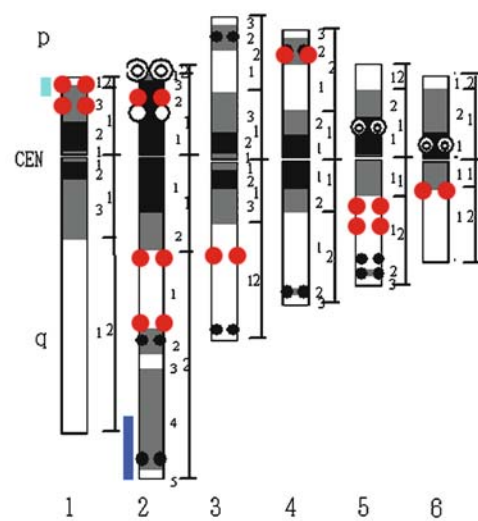
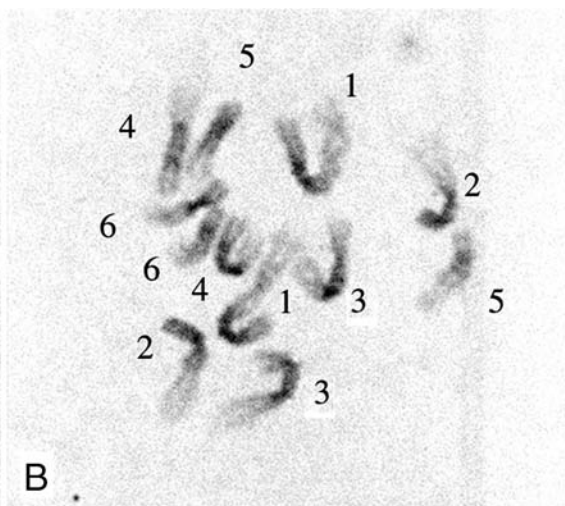
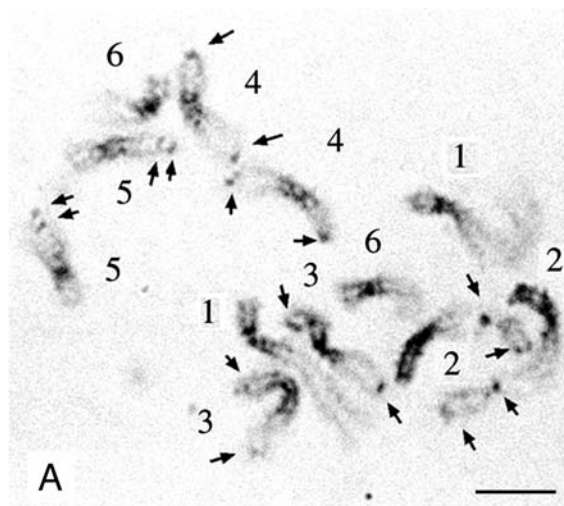


Figure 1. *Glycine max* chromosome at metaphase (A) and prometaphase (B). The scale bar indicates 5 µm. C: Idiograms of the soybean chromosomes that correspond to the five groups based on their condensation patterns. The arrowhead on the Group A chromosome indicates the position of the nucleolus organizing region. The condensation pattern is not presented for Group D because it varies even among chromosomes of this group. D: FISH mapping of 5S (green) and 45S rDNA (red) on prometaphase and metaphase chromosomes of annual progenitor, *G. soja*.

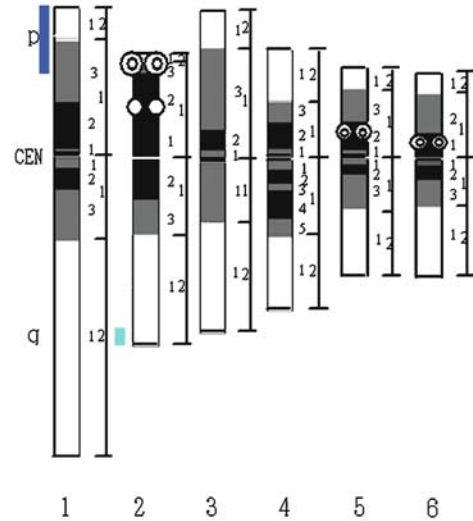
2006). For integration of the linkage groups and the chromosome map, 28 transformation-competent artificial chromosome (TAC) clones of the genetic map (<http://www.kazusa.or.jp/lotus/>) were localized by FISH using 5S and 45S rDNA as chromosomal landmarks. Figure 2C shows the somatic chromosome map of accession Miyakojima including the TAC clone positions. Pedrosa *et al.* (2002) compared data from FISH and genetic mapping for the accession

Gifu. BAC clones from 32 chromosomal regions of the six linkage groups were physically mapped to the six chromosomes.

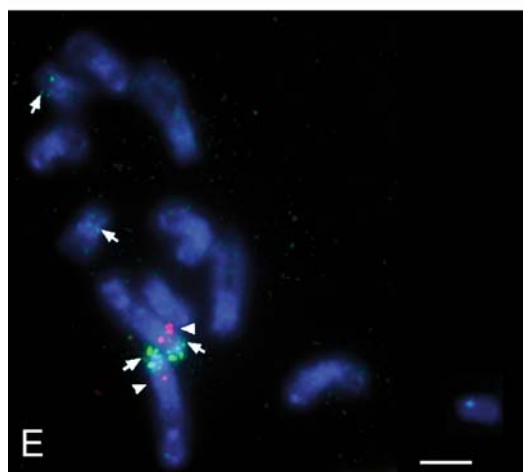
The two accessions differ particularly with respect to the size and morphology of chromosomes 1 and 2 (Figures 2C, 2D). Chromosome 1 is submetacentric and the largest chromosome of Gifu, while the homologous chromosome 1 is acrocentric and represents the second-largest chromosome in the accession



C Miyakojima



D Gifu



E

Miyakojima. A terminal NOR is located on the largest, submetacentric Miyakojima chromosome 2. In the accession Gifu the homologous chromosome 2 is smaller than chromosome 1. The two accessions were used as the parents to generate an F_2 mapping population for genetic analysis (Hayashi *et al.* 2001). A comparison of two genetic maps (Gifu $\times$ Miyakojima and *L. filicaulis* $\times$ Gifu) revealed a reciprocal translocation between Gifu and Miyakojima (Figures 2C, 2D). This translocation was confirmed cytogenetically. TAC clones which were assigned to linkage group 1 (within a region corresponding to the distal part of the short arm of Gifu chromosome 1), were localized at the distal region of the long arm of chromosome 2 of Miyakojima by FISH (Hayashi *et al.* 2001).

Kulikova *et al.* (2001) constructed a map of the pachytene chromosomes of *M. truncatula* ($2n=16$) using FISH with tandem repeats and 20 BAC clones. The pachytene chromosomes were identified by their length, centromere position, heterochromatin patterns and the positions of three repetitive sequences (5S and 45S rDNA, and the MtR1 tandem repeat). All *Medicago* chromosomes have characteristic heterochromatic blocks at the pericentromeric region occupied by three major repeats (either MtR1, R2, or R3) (Kulikova *et al.* 2004). Macrosynteny has been reported comparing the six chromosomes of *L. japonicus* with the eight chromosomes of *M. truncatula* (Choi *et al.* 2004). A recent genome comparison between *M. truncatula* and *L. japonicus* revealed 10 large-scale synteny blocks frequently covering entire chromosome arms and similar and homogeneous gene density within the euchromatin, although the abundant *Medicago* retrotransposons cause a 20–30% larger gene space compared to that of *Lotus* (Cannon *et al.* 2006).

Integrated chromosome maps for *Trifolium pratense*

Red clover (*T. pratense* L.) is an important forage legume that is widely cultivated in most temperate regions. It is an allogamous diploid ($2n=14$) with a

468 Mb genome (Arumuganathan & Earle 1991), which is similar in size to that of *L. japonicus*. The red clover genome is highly polymorphic due to its strong self-incompatible fertilization system. The high heterozygosity has hampered intensive genetic and genomic analyses. Recently, genetic maps based on microsatellite and EST markers were reported (Isobe *et al.* 2003, Sato *et al.* 2005).

A chromosome map of red clover was developed (Sato *et al.* 2005) based on the CP of mitotic prometaphase chromosomes which ranged in lengths from 5.1 to 7.4 μm . The prometaphase chromosomes provided a better spatial resolution than metaphase chromosomes, which ranged from 1.9 to 2.9 μm in length and this made it impossible to distinguish all seven chromosomes individually (Taylor & Chen 1988).

The 28S and 5S rDNA loci detected by FISH served as landmarks for integration of physical and genetic maps. The 28S rDNA locus on chromosome 1 revealed the most intense FISH signal, and less intense signals were detected in the proximal region of the short arm of chromosome 6. An additional signal was observed on one homologue of chromosome 5 in the accession HR (Figure 3A) but not in accession R130 (Figure 3B). One 5S rDNA locus occurred in the proximal region of the bottom arm of chromosome 1 adjacent to the NOR and two loci were detected on the short arm of chromosome 2 (Figure 3C) in both accessions.

The nuclear genome size was calculated to be 446 Mb for accession HR and 436 Mb for accession R130. The slightly but consistently larger genome size of HR, although not statistically significant, might be due to the high level of heterozygosity as to the rDNA locus on chromosome 5. The haplotype regarding the chromosome constitution as exemplified for chromosome 5 could be a cause of linkage distortion found by Sato *et al.* (2005).

To integrate the genetic linkage map and the chromosome density map, each linkage group was assigned to the corresponding chromosome after FISH with BAC clones specific to each linkage group (Sato *et al.* 2005). Microsatellite markers located close to

Figure 2. Cytological analysis of *Lotus japonicus* (Regel) Larsen, accessions Miyakojima and Gifu according to Ito *et al.* (2000). **A:** Prometaphase chromosomes of accession Miyakojima stained with Giemsa. Arrows indicate constitutive heterochromatin. **B:** Mitotic prometaphase chromosomes of accession Gifu stained with Giemsa. **C,D:** Chromosome density maps of accessions Miyakojima (**C**) and Gifu (**D**). The positions of the 5S rDNA (single circles); 45S rDNA (double circles); faint, unstable, small condensed (FUSC) regions (solid circles); and TAC clones (red circles) are shown. Light and dark blue lines mark the regions translocated between Miyakojima and Gifu. **E:** 45S rDNA (green) and 5S rDNA (red) were detected in pericentromeric regions. The bar indicates 5 μm .

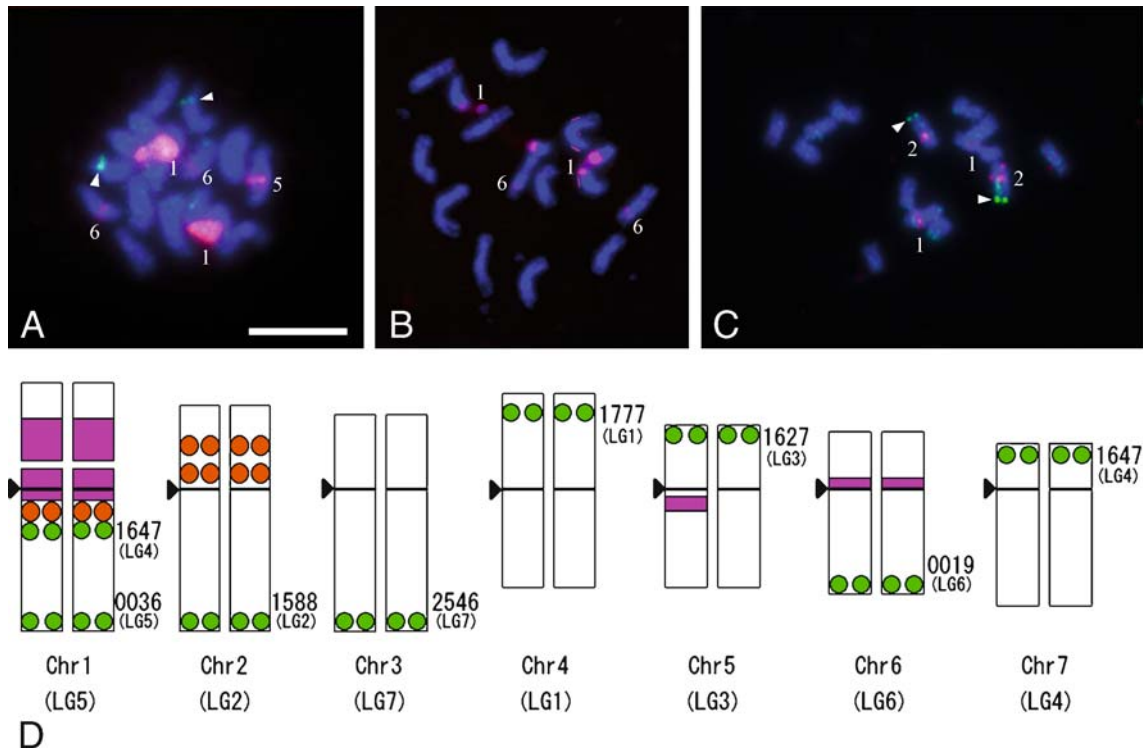


Figure 3. Cytological analysis of red clover. **A:** FISH signals for RCS1777 (green) and 28S rDNA (red) on chromosomes of accession HR stained with DAPI. Numbers indicate 28S rDNA loci on chromosomes 1, 5, and 6. The bar indicates 10 μ m. **B:** FISH signals for 28S rDNA (red) on chromosomes 1 and 6 of accession R130. **C:** FISH signals for RCS1588 (green) and 5S rDNA (red, indicated by chromosome numbers 1 and 2) in accession R130. **D:** Chromosome map of red clover. Green circles represent loci of seven BAC harboring linkage group-specific microsatellite markers; red boxes, 28S rDNA loci; and orange circles, 5S rDNA loci. The 28S rDNA locus of chromosome 5 is detected in accession HR but not in accession R130. Arrowheads indicate the centromere positions.

the end of each linkage group [RCS1777 (LG1), RCS1588 (LG2), RCS1627 (LG3), RCS1647 (LG4), RCS0036 (LG5), RCS0019 (LG6), and RCS2546 (LG7)] were used to test whether the linkage maps covered the entire chromosomes. The genomic BAC libraries were screened by PCR for the presence of these microsatellites. The positive BAC clones were used for FISH and gave signals according to the map position of the microsatellites exclusively at distal the region of one chromosome each (Figure 3A, 3C), except for RCS1647 that was detected at the short arm end of chromosome 7 and on chromosome 1 close to the NOR. The signal on chromosome 1 might be due to duplication of the corresponding chromosomal segment. The chromosomal position of markers used to assign the seven linkage groups to individual chromosome pairs are indicated in Figure 3D. The density of the markers varied among the seven linkage groups (Sato *et al.* 2005). LG5 with 28S rDNA

corresponds to chromosome 1 that harbors a large satellite at its short arm. The extended 28S rDNA region might implicate a short genetic distance because genetic recombination is usually not detectable between tandem repeats. LG5 represents a small linkage group with a low marker density, although it belongs to the largest chromosome. The small number of markers may reflect a lower gene density in this chromosome, but sequence conservation might contribute to the low degree of polymorphism and thus to the smallness of this linkage group.

Conclusions

The quantification of chromosome density by CHIAS, *in-situ* localization of repetitive sequences and high-resolution mapping of genes and/or markers by FISH are expected to facilitate the analysis of

gene density, segment duplication and other chromosome rearrangements and to yield integrated maps for legumes. Especially probes applicable for *Lotus*, red clover and other legumes will help to develop a framework for a common genomics of legumes. Molecular cytogenetics may contribute to this goal, as e.g. in the case of rice and tomato (Cheng *et al.* 2001, de Jong *et al.* 1999). From integration of linkage data, chromosome density and physical localization of DNA markers and/or genes basic research as well as legume breeding will benefit.

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