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The molecular cytogenetics of *Vigna unguiculata* (L.) Walp: the physical organization and characterization of 18*s*-5.8*s*-25*s* rRNA genes, 5*s* rRNA genes, telomere-like sequences, and a family of centromeric repetitive DNA sequences

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Abstract A knowledge of genome organization is important for understanding how genomes function and evolve, and provide information likely to be useful in plant breeding programmes involving hybridization and genetic manipulation. Molecular techniques, including in situ hybridization, molecular cloning and DNA sequencing, are proving valuable tools to investigate the structure, organization, and diversity of chromosomes in agricultural crops. Heterologous labelled 18*s*-5.8*s*-25*s* (pTa71) and 5*s* rDNAs (pTa794) were used for in situ hybridization on *Vigna unguiculata* (L.) Walp. chromosomes. Hybridization with 18*s*-5.8*s*-25*s* rRNA gene probes occurred at the same chromosomal sites which were positive to the CMA fluorochrome. Silver staining of nucleolar-organizing regions indicated that all the rDNA sites detected using the 18*s*-5.8*s*-25*s* rRNA gene probe possessed active genes. Degenerate telomeric repeats gave hybridization signals at the telomeres of most chromosomes and no intercalary sites were detected at metaphase; the sequences appear to have no preferential distribution in interphase nuclei. A repetitive *Dra*I family from *V. unguiculata* was cloned (pVuKB1) and characterized. The *Dra*I repeat is 488 nucleotides long, AT rich (74%), and hybridized on all chromosomes in the centromeric areas. The presence of this sequence family was investigated by Southern hybridization in different *Vigna* species and other *Leguminoseae*. It was only detected in *V. unguiculata*, and hence represents a species-specific DNA sequence.

Key words rDNA sites · Centromeric repetitive DNA · Telomere · In situ hybridization · Southern hybridization · Ag-NOR · Cowpea · Physical maps

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Introduction

The genus *Vigna* includes several crops of economic importance in Africa and parts of Asia. There is considerable interest in the mapping of genes for agronomic or nutritional characteristics (Young 1992), and interspecific hybridization has been attempted to expand the gene pool available to breeders (Fatokun and Singh 1987; Chen et al. 1989). The genus *Vigna*, along with the major agricultural genera *Glycine*, *Phaseolus* and *Cajunus*, is in the tribe *Phaseoleae* of the sub-family *Papilionoideae* in the *Leguminoseae* (*Fabaceae*) and hence is well placed to both donate and accept genes from a wide range of important crops with high protein quality and no requirement of nitrogenous fertilizers.

It is likely that either the use of integeneric hybrids or more directed manipulation of genes by transformation methods will enable useful genes to be transferred between different legumes and perhaps further. This process will be considerably assisted by both the use of extensive, cross-species, gene maps and by a knowledge of the cytogenetics of the species. A knowledge of the physical organization of the chromosomes and the physical, as well as genetical, locations of genes and other sequences along the chromosomes is likely to prove useful for directing selection programmes and for manipulation of chromosomes to give novel recombinants.

Molecular cytogenetic investigations are proving valuable to examine the structure, organization, and diversity of chromosomes in agricultural crops (Heslop-Harrison and Schwarzacher 1993) including wheat (Friebe et al. 1992; Schwarzacher et al. 1992; Mukai et al. 1993), barley (Leitch and Heslop-Harrison 1992, 1993) and their relatives in the *Triticeae*, tobacco (Kenton et al. 1993), and crucifers (Maluszynska and Heslop-

Harrison 1993a). These methods, based on fluorescent staining and in situ hybridization of various repetitive sequences, are particularly applicable to species with small chromosomes, such as *Brassica* or *Citrus* (Maluszynska and Heslop-Harrison 1993a; Roose et al., in preparation), where standard chromosome stains give only limited morphological differentiation.

The chromosomes of *Vigna* species are very small (Sen and Bhowal 1960); nevertheless, those of *V. unguiculata* (2n = 2x = 22) have been studied by C-banding as well as fluorochrome staining (Galasso et al. 1992, 1993). These studies indicated that there was considerable differentiation of the DNA along the chromosomes, several areas being enriched in GC-rich sequences. Chromosome bands ('heterochromatin'), detected by fluorochrome or C-banding, often consist of repetitive sequences in a tandem organization (satellite DNA families; see Pardue and Gall 1970). Repetitive DNA, consisting of motifs ranging from 2 to 10 000 base pairs long, repeated many hundreds or thousands of times, makes up a high proportion of many plant genomes. With the exception of a few such sequences, such as the rDNA and the telomeric repeats (see below), most have no known function. Following cloning of individual repetitive sequences, the combination of in situ hybridization, to discover the location along chromosomes, together with molecular analysis using Southern hybridization and sequencing, enables us to build a comprehensive picture of the characteristics of individual repetitive DNA sequence families. This knowledge is useful for the examination of chromosome structure and diversification. (Charlesworth et al. 1994; Nowak 1994; Schmidt and Heslop-Harrison 1994; Harrison and Heslop-Harrison 1995).

The 18 *s*-5.8 *s*-25 *s* ribosomal RNA genes (together referred to as 18 *s*-25 *s* rDNA below) and the unlinked 5 *s* rRNA genes (5 *s* rDNA), are normally present as many tandemly repeated units of genes and intergenic spacers at one or more pairs of sites in the genome, and can be readily localized by in situ hybridization. Changes in rDNA repeat copy number at single loci can be rapid and hence the study of rDNA copy number and site distribution is of interest of the examination of species relationships and evolution (Mukai et al. 1991; Maluszynska and Heslop-Harrison 1993a; Castilho and Heslop-Harrison 1995). Fluorescent in situ hybridization to chromosome preparations using labelled DNA probes is a key method for mapping both 18 *s*-25 *s* rDNA and 5 *s* rDNA sequences (Heslop-Harrison 1991), because it is not affected by the presence of variants within loci nor by differences in copy number between loci. Since the genes are highly conserved over all higher organisms, the use of heterologous sequences as probes reduces the chance of hybridization to intergenic spacer sequences, which may have homologous sites at many genomic locations.

Richards and Ausubel (1988) have characterized the telomeric repeat $(TTTAGGG)_n$ from *Arabidopsis thaliana*. This simple repetitive sequence hybridizes to the ends of the chromosomes of many plant species (Ganal et al. 1991; Schwarzacher and Heslop-Harrison 1991; Schubert et al. 1992; Wu and Tanksley 1993). Moreover, some plants have both terminal and intercalary locations of the sequence, which may be useful both for chromosome identification and for identifying karyotype rearrangements in plants (Schwarzacher and Heslop-Harrison 1991; Fuchs et al., 1995; Doudrick et al., in preparation) and animals (Scherthan 1990).

We initiated the present study to examine the chromosomes of cultivated *V. unguiculata* (cowpea) using molecular cytogenetic methods. In particular, we aimed to examine chromosome morphology and evolution, to localize the 18 *s*-25 *s* and 5 *s* rDNA and the transcriptional activity of the 18 *s*-25 *s* rRNA genes, to identify the locations of the telomeric repeats, and to isolate, characterize and localize major non-ribosomal repetitive DNA sequences both to define their significance in the evolution of the species and their potential use as chromosome markers.

Materials and methods

Plant material

The seeds of *Vigna* species used for this study were obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria, while seeds of other species belonging to different genera were obtained from commercial sources (Table 1).

Table 1 List of the materials used in the present study

Tribe	Genus	Subgenus	Section	Species	Accession
Phaseoleae	*Vigna*	*Vigna*	*Catiang*	*V. unguiculata* (L.) Walp.	Tvx 3236
Phaseoleae	*Vigna*	*Vigna*	*Catiang*	*V. unguiculata* (L.) Walp.	Commercial variety
Phaseoleae	*Vigna*	*Vigna*	*Vigna*	*V. luteola* Bentham	Tvnu 172
Phaseoleae	*Vigna*	*Vigna*	*Vigna*	*V. oblongifolia* A. Richard	Tvnu 135
Phaseoleae	*Vigna*	*Vigna*	*Vigna*	*V. ambacensis* Baker	Tvnu 755
Phaseoleae	*Vigna*	*Plectotropis*	–	*V. vexillata* A. Richard	Tvnu 72
Phaseoleae	*Glycine*	–	–	*G. max* (L.) Merr.	Commercial variety
Phaseoleae	*Phaseolus*	–	–	*P. coccineus* L.	Commercial variety
Vicieae	*Vicia*	–	–	*V. faba* L.	Commercial variety
Vicieae	*Pisum*	–	–	*P. sativum* L.	Commercial variety
Vicieae	*Lupinus*	–	–	*L. luteus* L.	Commercial variety

Chromosome preparation and staining

The seeds were germinated on moist filter paper for several days at 22–25 °C. Root-tips were treated with 2 mM of 8-hydroxyquinoline or with a saturated solution of paradichlorobenzene for 2 h before fixation in 100% ethanol:acetic acid (3:1) in order to accumulate metaphases. Chromosome preparation was carried out using the method of Schwarzacher et al. (1989). Briefly, fixed roots were washed in 0.01 M citric acid-sodium citrate, pH 4.6, buffer to remove fixative, and then digested in 2% (w/v) cellulase in 20% (v/v) pectinase for 40 min at 37 °C. The softened root-tips were again washed in the same buffer before squashing. One root-tip per slide was squashed in 45% acetic acid under a coverslip. The coverslip was removed after freezing on dry ice and the slides were allowed to dry in air before use.

Prior to in situ hybridization, some slides were stained with Chromomycin A3 (CMA) according to the technique of Schweizer (1976); after observation the slides were de-stained and used for in situ hybridization.

For the identification of active ribosomal sites precipitation of silver from $AgNO_3$ solution was utilized, following the technique of Bloom and Goodpasture (1976).

DNA extraction, digestion and cloning

Total genomic DNA of cowpea was isolated from 0.5–1.0 g of fresh leaves according to the procedure of Dellaporta et al. (1983). DNA was digested with the restriction endonucleases *Alu*I, *Rsa*I, *Dra*I, *Taq*I, *Xba*I, *Xbo*I, *Sma*I, *Hpa*II, *Msp*I, *Bam*HI, *Hae*III, *Hin*dIII and *Apa*I. The resulting fragments were electophoretically separated on a 1% agarose gel. After digestion with *Dra*I, a distinct restriction fragment about 500 bp long was observed; it was cut from the gel, purified and cloned in pUC18 in *E. coli* strain DH 5α. One recombinat clone, pVuKB1, was used for in situ hybridization to chromosome preparations, and for Southern analysis. It was sequenced on an automated sequencer (Pharmacia ALF) using the dideoxy chain-terminator procedure. Homologies with other sequences were sought in the Genbank (release 84) and EMBL (release 39) database.

DNA probe labelling and fluorescent in situ hybridization

The following probes were used for in situ hybridization:
pTa71 contains a 9-kb *Eco*RI fragment including the 18 *s*-5.8 *s*-25 *s* rRNA genes and intergenic spacer regions, isolated from *Triticum aestivum* (Gerlach and Bedbrook 1979), and was labelled with tetramethyl rhodamine isothiocyanate (TRITC) by nick-translation.
pTa794 corresponds to a complete 410-bp 5 *s* gene unit, containing the 5 *s* gene and the intergenic spacers, isolated from *T. aestivum* (Gerlach and Dyer 1980) and labelled with digoxigenin-11-dUTP using the polymerase chain reaction.
pVuKBI, a 488-bp *Dra*I fragment isolated from *Vigna unguiculata* Tvx3236 labelled with digoxigenin-11-dUTP using the polymerase chain reaction.
A degenerate telomere sequence was made using the methods of Ijdo et al. (1991). Briefly, the sequences $(TTTAGGG)_6$ $(CCCTAAA)_6$ were mixed together and labelled with biotin-11-dUTP in a PCR buffer.

For in situ hybridization, slides were pre-treated with 100 µg/ml of RNase A in 2 × SSC for 1 h at 37 °C and washed twice in 2 × SSC. After incubation with 10 µg/ml of pepsin in 10 mM HCl for 10 min at 37 °C, chromosome preparations were stabilized in freshly de-polymerized 4% (w/v) paraformaldehyde in water for 10 min, dehydrated in a graded ethanol series and air dried.

The hybridization mixture, consisting of 50–150 ng/µl of DNA probe, 50% (v/v) formamide (for pTa71, pTa794 and pVuKB1 probes) or 30% (v/v) (for the degenerate telomeric probe), 10% (w/v) dextran sulphate, 0.1% SDS and 300 ng/µl of sheared salmon sperm DNA in 2 × SSC, was incubated for 10 min at 70 °C and chilled on ice. Then 30 µl of hybridization mixture was added to the chromosome preparations and covered with a plastic coverslip. The hybridization mixture and the chromosomal DNA were denatured together at 70 °C for 5 min; the temperature was then gradually decreased to 37 °C using a Hybaid Omnislide temperature cycler (see Heslop-Harrison et al. 1991). Hybridization between target and probe was carried out at 37 °C overnight. After hybridization, the slides were given a stringent wash in 20% (v/v) formamide in 0.1 × SSC (for pTa71, pTa794 and pVuKB1 probes) or 1 × SSC (for telomeric probe) at 42 °C, to remove mismatched or unhybridized probe molecules (the wash removes sequences with less than 85% or 67% homology respectively), and slides were incubated in immunofloresecent reagents. For the detection of digoxigenin- or biotin-labelled probes, slides were equilibrated in 4 × SSC/0.1% (v/v) Tween 20 and blocked in 5% (w/v) bovine serum albumin in 4 × SSC/0.1% (v/v) Tween 20 for 5 min. Slides were incubated with 2 µg/ml of sheep anti-digoxigenin antibody conjugated with FITC or streptavidin Cy3 in a moist chamber at 37 °C for 1 h. Excess antibody was removed by washing the slides in 4 × SSC/0.1% (v/v) Tween 20 for 3 × 5 min. After counterstaining with DAPI (2 µg/ml), and sometimes with propidium iodide (1 µg/ml) when red fluorescing probes were not used, the slides were mounted in antifade solution (AF1, Citifluor). Slides were examined with Leitz or Zeiss epifluorescence microscopes with single and triple band-pass filters suitable for the fluorochromes being used. Photographs were taken on Fujicolor Super HG 400 colour print film, digitized to PhotoCD, and printed from Adobe Photoshop after contrast optimization using only functions affecting the whole imagte equally (i.e. no signals were individually enhanced nor background removed; the images are indistinguishable from those obtained by optimized printing and overlaying of the negatives).

Southern hybridization

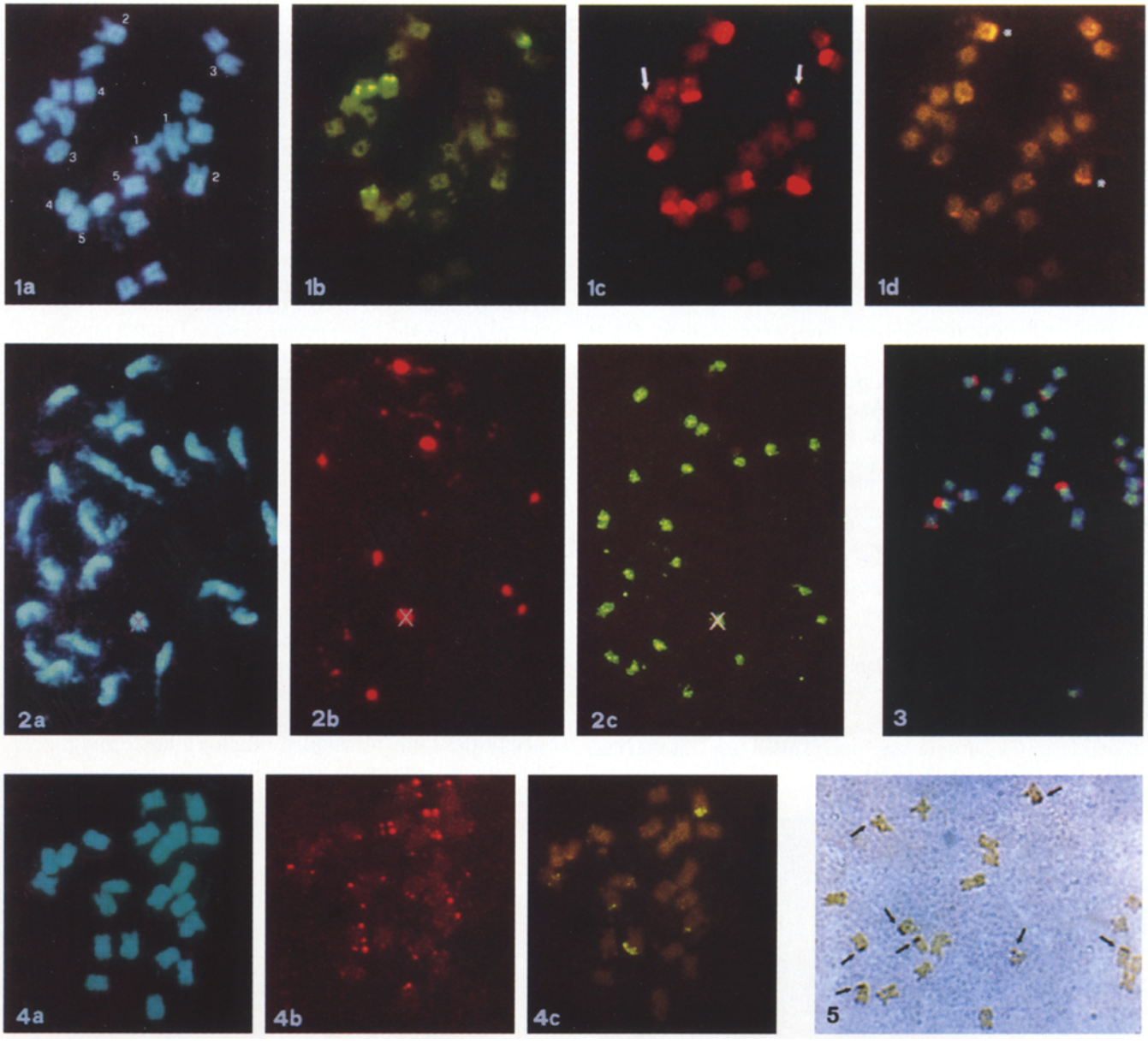
Hybridization of pVuKB1 to different species and genera (Table 1) was examined by Southern techniques using the ECL system (Amersham). Six to eight micrograms of genomic DNA were digested with *Dra*I, separated on a 1% agarose gel and transferred onto a nylon membrane. After hybridization using pVuKB1 as a probe, filters were washed with a stringency of 85%.

Results

The synchronization and spreading methods described enabled reliable preparation of metaphase (Figs 1, 3, 4, 5) and pro-metaphase (Fig. 2) spreads with 22 well-separated chromosomes. After CMA staining, *V. unguiculata* had four pairs of brightly fluorescing sites, one of which was approximately twice as strong as the other three, all in sub-telomeric positions (Fig. 1 d) in agreement with Galasso et al. (1993).

Localization of 18 *s*–25 *s* and 5 *s* rDNA

In situ hybridization using rhodamine-labelled 18 *s*–25 *s* rDNA showed that the probe was located on five pairs of chromosomes. The four major sites showing a bright signal were at sub-telomeric positions, on satellited chromosome pairs, while the fifth minor pair of sites was located at a centromeric region (Figs. 1 c, 2 b). Major sites of 5 *s* rDNA were located on two pairs of chromosomes (Fig. 1 b). One site was on the opposite arm from a 18 *s*–25 *s* rDNA site, while the other was on a chromosome without any 18 *s*–25 *s* signals (Figs. 1 b, c). No minor sites were detected. Among the four major sites of 18 *s*–25 *s* rDNA, one can be identified with the most brilliant CMA site on chromosome 2; the chromosome



Figs. 1–5 Chromosome preparations of *V. unguiculata*. (**1**) The same metaphase was photographed after DAPI (**1 a**) staining, in situ hybridization with a pTa794 probe (**1 b**) showing the 5 *s* rDNA sites, the pTa71 probe (**1 c**) corresponding to the 18 *s*–25 *s* rDNA clusters (minor sites arrowed) and CMA (**1 d**), the symbols * indicate the most fluorescent chromosome pair). (**2**) A pro-metaphase showing DAPI staining (**2 a**), in situ hybridization with a rhodamine-labelled clone pTa71 (**2 b**), and with a digoxigenin-labelled pVuKB 1 clone (**2 c**). (**3**) Double target in situ hybridization using the same probes as in **2b** and **2c**, demonstrating the centromeric labelling of the pVuKB 1 probe. (**4**) The chromosomes stained with DAPI (**4 a**) were subjected to in situ hybridization with a biotin-labelled $(TTTAGGG)_6$ $(CCCTAAA)_6$ telomeric probe (**4 b**) and a direct fluorescin-labelled pTa71 clone (**4 c**). Metaphase following silver staining (**5**), the *arrows* indicate active ribosomal sites.

showing sites of both the 18 *s*–25 *s* and 5 *s* rDNAs can be identified as chromosome 4; the remaining major 18 *s*–25 *s* rDNA sites can be associated with the satellited chromosome 3 and chromosome 5. The minor 18 *s*–25 *s* rDNA and the other 5 *s* rDNA locations lie on chromosomes which cannot be uniquely recognised using cytological features. For karyotype nomenclature refer to Galasso et al. (1992).

All four major pairs of 18 *s*–25 *s* rDNA sites showed a strong positive reaction with the Ag-NOR staining method (Fig. 5). The minor 18 *s*–25 *s* rDNA site did not show any Ag-NOR reaction.

Localization of the PCR-telomere oligonucleotide product

Hybridization of the PCR product was detected at most telomeres (Fig. 4b). The strength of hybridization, which was related to the copy number of the sequence, was highly variable, with some chromosomes having strong signals, while a few showed no detectable signal. Normally, a similar strength of hybridization was seen on both chromatids of a chromosome arm. No intercalary sites were detected at metaphase, whereas the sequence seemed to be distributed over most of the area of spread

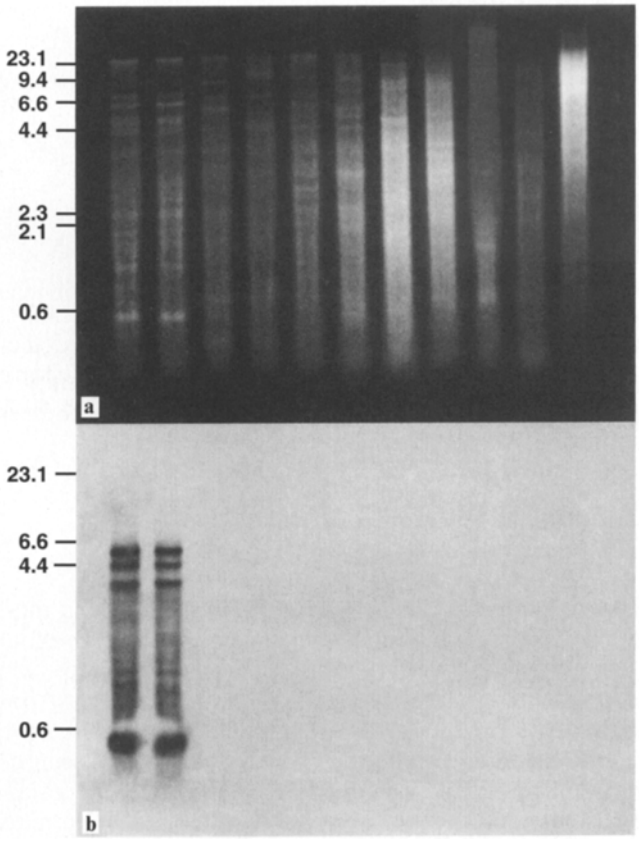
interphase nuclei. Sites were usually detected at the end of the chromosome arms, and double target hybridization with the 18 *s*–25 *s* rDNA and the telomeric probes showed that the telomere sequence intensity was reduced on the chromosomes carrying 18 *s*–25 *s* rDNA sequences (Figs. 4b,c).

*Dra*I repetitive DNA family

Sequence identification and cloning

Various restriction endonucleases were used to digest total genomic DNA of *V. unguiculata*. Several enzymes show bands of restriction fragments, indicative of the presence of repetitive DNA, and the enzyme *Dra*I yielded a particularly prominent restriction fragment, in the region of 500 bp (Fig. 6 a), which was cut from the gel and cloned. The probe was named pVuKB1 and used for both in situ and Southern hybridization and sequence characterization.

Fig. 6 Distribution of the *Dra*I sequence in the genus *Vigna* and other *Leguminosae*. Ethidium bromide-stained gel showing size-fractionated *Dra*I-digested genomic DNA of (left to right, except first lane λ DNA/*Hin*dIII fragments) *V. unguiculata* Tvx 3236, *V. unguiculata* commercial variety, *V. luteola*, *V. oblongifolia*, *V. ambacensis*, *V. vexillata*, *Glycine max*, *Vicia faba*, *Phaseolus coccineus*, *Lupinus luteus*, *Pisum sativum* (**6 a**); after transferring onto nylon membrane, the filter was hybridized with pVuKB 1 (**6 b**)



In situ hybridization

Digoxigenin-11-dUTP-labelled pVuKB 1 was hybridized to cowpea. The hybridization signal was detected in the centromeric areas of all chromosomes at both metaphase (Fig. 3) and prometaphase (Fig. 2 c). The signal sites corresponded to the locations of many C-bands.

Southern hybridization

Hybridization of pVuKB 1 to *Dra*I digests of DNA from several *Leguminoseae* species (Table 1) gave strong hybridization to *V. unguiculata*. After extended exposure, *V. vexillata* showed a signal near the limit of detection, but no hybridization was detected on tracks from the other species (Fig. 6 b).

Sequence analysis

Figure 7 shows the nucleotide sequence of pVuKB 1. No significant homologies were found with other sequences in the EMBL/Genbank database. This sequence is likely to represent a complete repeat unit since the same unit length is evident in digests with *Hae*III and *Hin*dIII, and degenerate recognition sites for these enzymes are present in the sequence. It is AT rich (74%) and 488 nucleotides long, unusually long for a tandemly repeated sequence, many of which have a repeat unit corresponding to a nucleosome (160–190 bp long, see, e.g. Kamm et al. 1994, 1995; Schmidt and Heslop-Harrison 1994). The sequence has an internal unit of a 24-bp consensus, AATCAAAGTAAAGTCTCAATTAAT, which is repeated no less than seven times (in either orientation), representing 35% of the total sequence. No similar motif is found in other sequences in the databases; the closest matches are in anonymous cosmids from *Cenorhabditis elegans*. Other features of the pVuKB 1 sequence, such as short inverted repeats, palindromes, and the stable folding predicted by the Zuker energy minimization methods in the GCG nucleotide-sequence analysis package, are generally not statistically significant as they are expected by chance alone in an AT-rich sequence.

Discussion

In situ hybridization shows that there are four major pairs of 18 *s*–25 *s* rDNA sites in *V. unguiculata* (Figs. 1–4), which correspond to the chromosomal regions giving a CMA-positive reaction. The presence of these four sites was indicated previously by reactions to CMA and Giemsa staining (Galasso et al. 1993). A fifth centromeric pair of 18 *s*–25 *s* rDNA sites was detected by in situ hybridization (Figs. 1c, 2b). Since a heterologous probe from wheat was used, all sites are likely to include coding genes, and not variants of the intergenic spacer

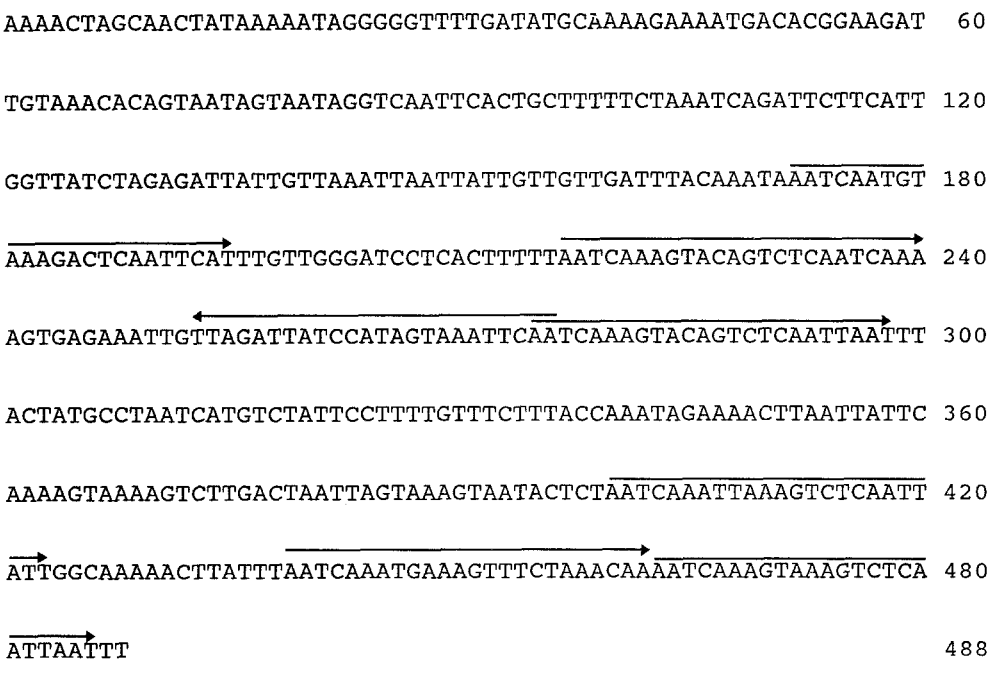


Fig. 7 Nucleotide sequence of the *V. unguiculata Dra*I repeat. Internal subrepeats are marked by *arrows*

region which an 18 *s*–25 *s* rDNA probe isolated from *V. unguiculata* itself might detect. The high resolution of double target in situ hybridization associated with CMA staining, has allowed us to identify the CMA-positive area on chromosome 3 with the secondary constriction on the sat-chromosome of cowpea which is positive to the 18 *s*–25 *s* rDNA. This is in contrast with a previous paper (Galasso et al. 1993) and demonstrates the need to employ high-resolution techniques in species with small chromosomes.

Silver staining of basic proteins associated with the expression of the 18 *s*–25 *s* rRNA genes showed that only the four pairs of major telomeric sites were expressed (Fig. 5), giving a maximum of eight nucleoli at interphase (data not shown), while no activity of the centromeric sites was detected in root-tip nuclei. Whether the site is ever expressed is unknown. However, in barley, evidence from the association of meiotic chromosomes with nucleoli indicates that the activity of many minor sites can be detected at this stage of plant development (Tsuchiya 1960); the meiotic assay may be more sensitive than silver staining, or perhaps the minor sites are only active at this critical stage of plant development. Two major pairs of 5 *s* rDNA sites were detected, one distal on the opposite arm to a 18 *s*–25 *s* rDNA site, and the other proximal on a chromosome without a 18 *s*–25 *s* rDNA site (Fig. 1 b, c).

Examination of rDNA is of considerable interest because the DNA sequence (particularly in the intergenic spacer region), the copy number of the repeat at each site, and the number of sites, can all evolve rapidly. Even in tissue culture, changes can be detected (Leitch et al. 1993), while changes during species evolution can be used in reconstructing phylogenies and detecting karyotype rearrangements. In the three diploid (as conventionally regarded) and the three tetraploid *Brassica* species derived from the diploids (U 1935; Quiros et al. 1986; see latter for discussions of ploidy levels), we found that the number of rDNA sites was reduced in the tetraploid (Maluszynska and Heslop-Harrison 1993 a). However, the most extensive studies have been made in the Triticeae. Comparisons of the sites of 5 *s* and 18 *s*–25 *s* rDNA in wheat, rye, barley and *Ae. umbellulata* on group-1 and group-5 chromosomes indicate that if each inversion or deletion/insertion event occurred only once, no simple phylogenetic tree can account for the relative rearrangements of the genomes (Castilho and Heslop-Harrison 1995). In contrast, studies of homoeology using RFLP maps of barley, wheat and rye, reveal only evolutionary translocations (Devos et al. 1993 a, b) and there are no major inversions, deletions or insertions.

In *Vigna*, it will be interesting to compare the distribution of rDNA sites and their evolutionary changes during the selection of cultivars and evolution in the genus. Molecular cytogenetic methods are able to detect some types of chromosomal rearrangements which are difficult to identify using genetic markers. rDNA sites may be useful as markers along chromosomes both to connect physical chromosomes with genetic linkage groups and to follow individual chromosomes through hybridization and backcrossing programmes.

V. unguiculata [synonym of *V. sinensis* (L.) Hassk., Ng and Maréchal 1985] has a DNA content similar to that of *V. radiata* (2n = 22) at 0.5 pg 1C or 480 Mbp (Bennett and Smith 1976; Bennett et al. 1982), and its chromosomes are about 50% bigger than those of *A. thaliana* (2n = 10, 150 Mbp, Heslop-Harrison and Schwarzacher 1991). Despite the small genome size of *Vigna* species, size fractionation of genomic DNA restriction enzyme digests reveals many bands, consisting of highly repeated restriction fragments (Fig. 6 and data not shown). C-

banding of chromosomes has demonstrated that heterochromatin is distributed at both centromeres and telomeres (Galasso et al. 1993). The probe pVuKB 1 is localised exclusively at centromeric sites, while many of the sites of telomeric heterochromatin co-localize with the 18 *s*–25 *s* rDNA sequences. Some of the sequences which give positive C-bands are the rDNA sequences themselves, but other repetitive sequences are often associated with the rDNA (Schweizer 1980).

The structure of heterochromatin has both theoretical and evolutionary implications. The architecture of heterochromatin tends to be conserved in related taxa, and fluorochrome and C-banding patterns of related taxa indicate that they have a similar chromosome structure (Zheng et al. 1993). However, among the species tested, the repeat unit pVuKB 1 is essentially specific to *V. unguiculata* (Fig. 6) showing only very weak hybridization to *V. vexillata*. Whether the limited cross hybridization is due to the presence of substantially diverged sequences with high copy number, or a similar sequence with very low copy number, is unknown. However, these data, along with the similarity in karyotype (Galasso et al. 1993), isozyme data (Vaillancourt and Weeden 1993), and RFLP analysis (Fatokun et al. 1993), provide evidence that *V. vexillata* is taxonomically close to *V. unguiculata*, in contrast to the conventional view based on morphology (Maréchal et al. 1978). Molecular cytogenetic investigations also lead to a reassessment of relationships among the small ephemeral species in the *Cruciferae* tribes *Sysimbrideae* and *Arabideae*, where it is clear that the species *A. thaliana*, conventionally placed in the former tribe, is closer to *Cardaminopsis arenosa*, conventionally placed in the latter, while other species currently in the genus *Arabidopsis* are much more remote (Kamm et al. 1995; Maluszynska and Heslop-Harrison 1993 b).

The products of the PCR amplification of telomere sequences give oligonucleotides with a degenerate sequence based on TTTAGGG and its complement (Doudrick et al., personal communication). In situ hybridization localized the sequence exclusively at the telomeres and, as in cereals (Schwarzacher and Heslop-Harrison 1991), considerable variation in copy number was found from chromosome end to chromosome end (Fig. 4). There were no high-copy number sites of intact or degenerate telomeric sequences elsewhere in the genome, contrasting to many other species (Fuchs et al. 1995). Interstitial loci of telomeric sequences in species such as *Vicia faba* have been taken to indicate chromosome fusion during karyotype evolution (Schubert et al. 1992).

Molecular cytogenetic investigations of plant genomes are helpful to combine information from molecular studies with knowledge about chromosome structure and morphology. The work described here provides a basis for building a physical map showing the locations of genes along the chromosomes of *V. unguiculata*. Furthermore, knowledge of the morphology of chromosomes and the repetitive sequences they carry can be useful in linking genetic and physical maps of a species, as well as in plant breeding programmes where the data can assist in the selection of lines with novel chromosome combinations derived from interspecific or intergeneric hybrids.

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