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Physical mapping of rRNA genes by fluorescent in-situ hybridization and structural analysis of 5S rRNA genes and intergenic spacer sequences in sugar beet (*Beta vulgaris*)

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Abstract A digoxigenin-labelled 5S rDNA probe (pTa-794) and a rhodamine-labelled 18S-5.8S-25S rDNA probe (pTa71) were used for double-target in-situ hybridization to root-tip metaphase, prophase and interphase chromosomes of cultivated beet, *Beta vulgaris* L. After in-situ hybridization with the 18S-5.8S-25S rDNA probe, one major pair of sites was detected which corresponded to the secondary constriction at the end of the short arm of chromosome 1. The two rDNA chromosomes were often associated and the loci only contracted in late metaphase. In the majority of the metaphase plates analyzed, we found a single additional minor hybridization site with pTa71. One pair of 5S rRNA gene clusters was localized near the centromere on the short arm of one of the three largest chromosomes which does not carry the 18S-5.8S-25S genes. Because of the difficulties in distinguishing the very similarly-sized *B. vulgaris* chromosomes in metaphase preparations, the 5S and the 18S-5.8S-25S rRNA genes can be used as markers for chromosome identification. Two *XbaI* fragments (pXV1 and pXV2), comprising the 5S ribosomal RNA gene and the adjacent intergenic spacer, were isolated. The two 5S rDNA repeats were 349 bp and 351 bp long, showing considerable sequence variation in the intergenic spacer. The use of fluorescent in-situ hybridization, complemented by molecular data, for gene mapping and for integrating genetic and physical maps of beet species is discussed.

Key words *Beta vulgaris* · Sugar beet · In-situ hybridization · rRNA genes · Intergenic spacer Physical mapping

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Introduction

Cultivars of *Beta vulgaris* L. are important crops grown for sugar production (sugar beet), animal feed (fodder beet), and as vegetables (table beet and Swiss chard). Crosses with wild beet relatives have been made with the aim of transferring economically important disease and nematode resistance genes into the crop (for review see Nakamura et al. 1991).

As in other crops, many cytological studies have been undertaken to support breeding programmes and provide general information about the cytogenetics of *B. vulgaris*. The karyotype of *B. vulgaris* consists of nine similarly-sized meta- to submeta-centric chromosome pairs which are very small (Bosemark and Bormotov 1971; de Jong 1981; Nakamura et al. 1991) as indicated by the haploid 1C genome size of 1.25 pg (1100 Mbp, Bennett and Smith 1976). Molecular cytogenetic studies have not yet been performed in this species, although the methods, in particular the use of fluorochrome staining and fluorescent in-situ hybridization, are known to be extremely valuable for the investigation of species with small or similarly-sized chromosomes (e.g., *Arabidopsis thaliana* and *Brassica* species, Maluszynska and Heslop-Harrison 1991, 1993), for the localization of genes along chromosomes (Mukai et al. 1990; Leitch and Heslop-Harrison 1992, 1993; Lehfer et al. 1993), and for the examination of the behaviour of chromosomes transferred from alien species (Schwarzacher et al. 1992).

In the present paper, we have applied fluorescent in-situ hybridization to the genome analysis of cultivated beet. As a first step towards the physical mapping of sugar beet chromosomes, we localized the genes for the 18S-5.8S-25S rRNA and 5S rRNA. The cytological investigations have been accompanied by molecular analyses of the 5S rDNA repeats. Both the 18S-5.8S-25S rRNA and 5S rRNA genes are present as multigene families organized in long tandem arrays and their distribution and expression has been studied in detail in several plant species (Flavell 1986; Rogers and Bendich 1987). Because of their universal oc-

currence and redundancy, the ribosomal genes are a valuable model for karyotype analysis and are also useful for comparative studies of genome organization.

Materials and methods

Cultivated beet root-tips were prepared from seedlings of *B. vulgaris* cv Rosamona (2n=18), a commercial cultivar used for fodder and sugar production. Seeds were germinated on moist filter paper for 72 h at 25 °C. For synchronization of cell divisions, germinating seeds were kept at 4–5 °C for 24 h, and then transferred to 25 °C for 22–26 h. Root-tips were treated with 2 mM 8-hydroxyquinoline for 3–5 h and fixed in methanol:glacial acetic acid (3:1).

Heterologous ribosomal DNA sequences were used as probes for in-situ hybridization. Clone pTa71 (Gerlach and Bedbrook 1979) contains a 9-kb *Eco*RI fragment of *Triticum aestivum* L. consisting of the 18S-5.8S-25S rRNA genes and the transcribed and non-transcribed intergenic spacer regions. Clone pTa794 (Gerlach and Dyer 1980) contains part of the *T. aestivum* 5S rRNA gene and spacer sequences. pTa71 was labelled with rhodamine-4-dUTP (Amersham) by nick translation, while pTa794 was labelled with digoxigenin-11-dUTP (Boehringer Mannheim) using the polymerase chain reaction.

Chromosome preparation was carried out using the method of Schwarzacher et al. (1989). Briefly, fixed root-tips (2–3 mm long) were washed in enzyme buffer (10 mM citric acid/sodium citrate, pH 4.6) for 20 min and digested in 2% cellulase (Calbiochem) and 20% pectinase from *Aspergillus niger* (Sigma) in enzyme buffer for 70–90 min at 37 °C. 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 preparation was air dried. Slides were incubated for several hours at 37 °C before use. For in-situ hybridization, slides were pretreated with 100 µg/ml of RNase A in 2× SSC (0.3 M NaCl, 0.03 M sodium citrate) for 1 h at 37 °C and washed twice in 2× SSC. After incubation with 40 units/ml of pepsin (Sigma) in 10 mM HCl for 10 min at 37 °C, chromosome preparations were stabilized in freshly-depolymerized 4% (w/v) paraformaldehyde in water for 10 min, dehydrated in a graded ethanol series and air dried. In-situ hybridization was carried out according to Heslop-Harrison et al. (1991). The hybridization solution, consisting of 0.5–1.5 ng/µl of DNA probe, 50% (v/v) formamide, 10% (w/v) dextran sulphate, 0.1% (w/v) SDS (sodium dodecyl sulphate) and 300 ng/µl sheared salmon sperm DNA in 2× SSC, was incubated for 10 min at 70 °C and chilled on ice. Thirty microliters of hybridization mixture were added to each chromosome preparation and covered with a plastic coverslip. The hybridization mixture and the slides were denatured together at 65–70 °C in a modified thermocycler for 5 min, and the temperature was then gradually decreased to 37 °C. Hybridization was carried out in a humid chamber at 37 °C overnight. After hybridization the slides were washed for 10 min each in 2× SSC at room temperature, in 20% (v/v) formamide in 0.1× SSC at 42 °C, 2× SSC at 42 °C and 2× SSC at room temperature. For the detection of digoxigenin-labelled probes, slides were equilibrated in detection buffer [4× SSC/0.2% (v/v) Tween 20] and blocked in 5% (w/v) bovine serum albumin in detection buffer for 5 min before incubation with 2 µg/ml FITC (fluorescein isothiocyanate)-conjugated sheep anti-digoxigenin antibody (Boehringer Mannheim) in a humid chamber at 37 °C for 1 h. Excess antibody was removed by washing the slides in detection buffer for 3×5 min. After counterstaining with DAPI (4′,6-diamidino-2-phenylindole, 2 µg/ml) or propidium iodide (1 µg/ml), the slides were mounted in antifade solution (AF1, Citifluor). Slides were examined with a Leitz epifluorescence microscope with filter sets A, I2/3 and an Omega triple-band passfilter set with UV block. Photographs were taken on Fujicolor Super HG 400 colour print film.

DNA extraction, restriction enzyme digestion, cloning into pBSK$^+$ and selection of recombinant clones were performed as described previously (Schmidt et al. 1991). Both strands of the inserts were sequenced on an automated sequencer (Pharmacia) using the dideoxy chain-termination procedure. For sequence-data processing the GCG computer package (FASTA) was used. Sequences appear in the EMBL data library under the accession numbers Z25803 and Z25804.

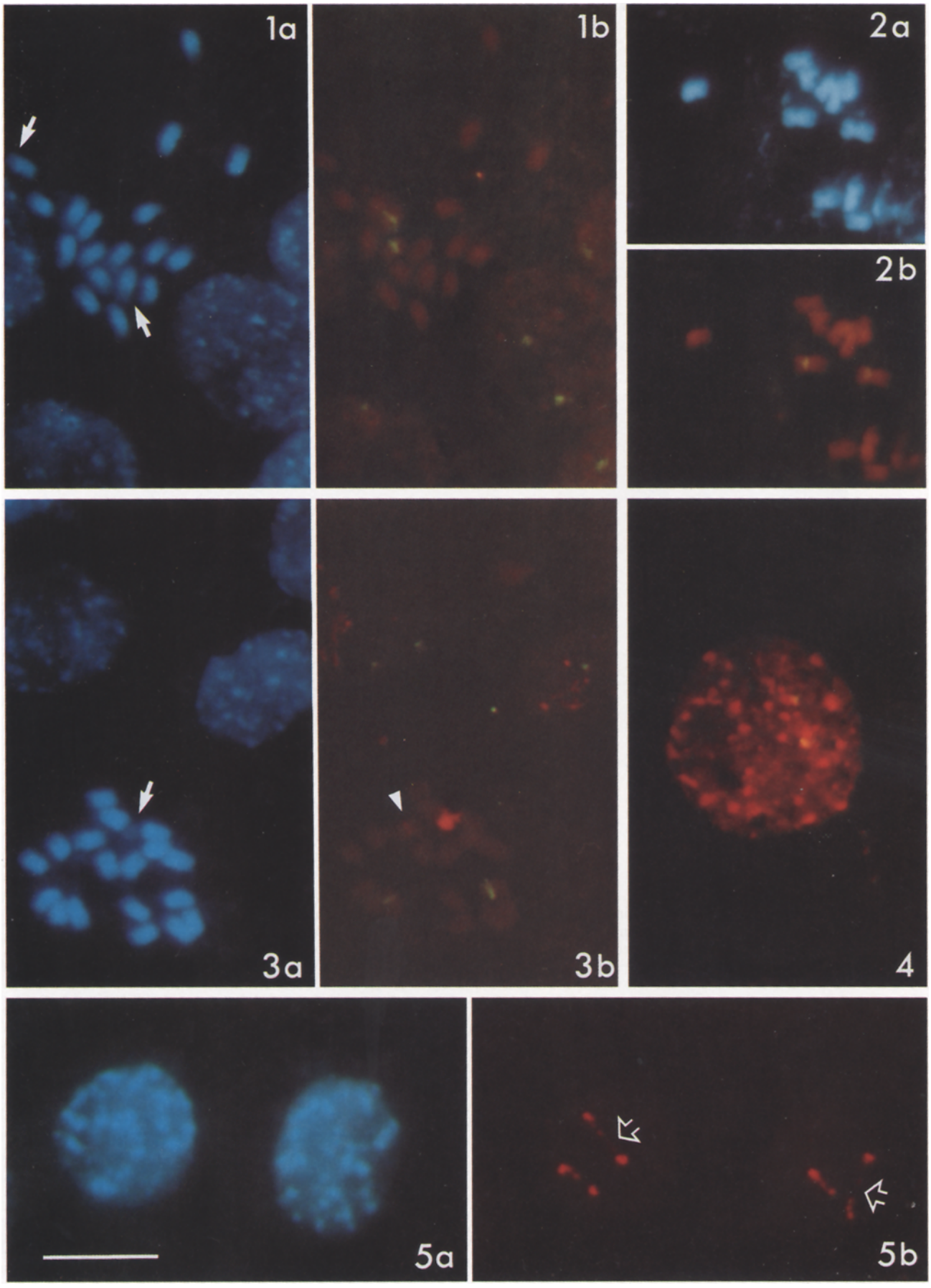
Results

Figures 1–7 show micrographs of root-tip chromosomes and nuclei from *B. vulgaris* after fluorescent in-situ hybridization and counterstaining. The synchronization of cell divisions and the enzymatic-spreading method described enabled reliable preparation of chromosome spreads with many complete pro-metaphases and metaphases. The pepsin pretreatment was essential to obtain chromosomes and nuclei free from debris and cytoplasm. The chromosomes maintained their morphology (Figs. 1 a, 2 a and 3 a) and most chromosomes, particularly at pro-metaphase and prophase (e.g., Fig. 7 a), showed strongly DAPI-stained regions near their centromeric constrictions.

Localization of 18S-5.8S-25S rRNA genes

After in-situ hybridization with clone pTa71 (labelled with rhodamine-4-dUTP), three hybridization sites were detected on metaphase spreads (Figs. 3 b and 6 b). The two strongest signals, showing the site of the major 18S-5.8S-25S rRNA gene clusters, were present on a large pair of chromosomes, normally associated with a secondary constriction at the rDNA site at the end of the small arm. The nucleolus-organizing chromosomes can also be identified after DAPI staining by the lower intensity of staining at the site of the rDNA signal (compare Figs. 3 b and 6 b with 3 a and 6 a, respectively; see also Fig. 1 a). The presence of the single additional site was analyzed on spreads made from four different root-tips, each from a different monogerm seed. In total, 24 complete early and late metaphases

Figs. 1–5 Fluorescent in-situ hybridization to root-tip metaphase chromosomes and interphase nuclei of *B. vulgaris* (2n=18). **1a**, **2a**, **3a**, **5a**. The DNA stain DAPI (blue fluorescence) shows chromosome or nuclear morphology. The terminal NORs of chromosome pair 1 are stained more weakly (*arrow*). **1b**, **2b**, **4**. Localization of 5S rRNA gene clusters. Two metaphase chromosomes show signals of yellow-green fluorescence on the short arm near the centromere of one of the largest chromosomes corresponding to one pair of 5S rDNA loci. Two 5S rRNA sites are visible at interphase. A heterologous 5S rRNA probe (pTa 794) from wheat was labelled with digoxigenin and detected by fluorescein-conjugated anti-digoxigenin. DNA was counterstained with propidium iodide (orange fluorescence). **3b** Simultaneous localization of 18S-5.8S-25S rRNA genes (direct rhodamine-labelled clone pTa71) and 5S rRNA genes (digoxigenin-labelled clone pTa794). Exposure with a multiband passfilter shows one pair of 5S rRNA gene clusters (yellow-green fluorescence) in each cell and one major pair of 18S-5.8S-25S rRNA genes (red fluorescence). The 18S-5.8S-25S rRNA sites are condensed and associated with each other. The *arrowhead* indicates the single minor site of the 18S-5.8S-25S rRNA genes. **5b** Red fluorescence shows one pair of major 18S-5.8S-25S rRNA gene clusters in each interphase nucleus. Decondensed rDNA chromatin is indicated by *open arrows*. Bar 10 µm


1a
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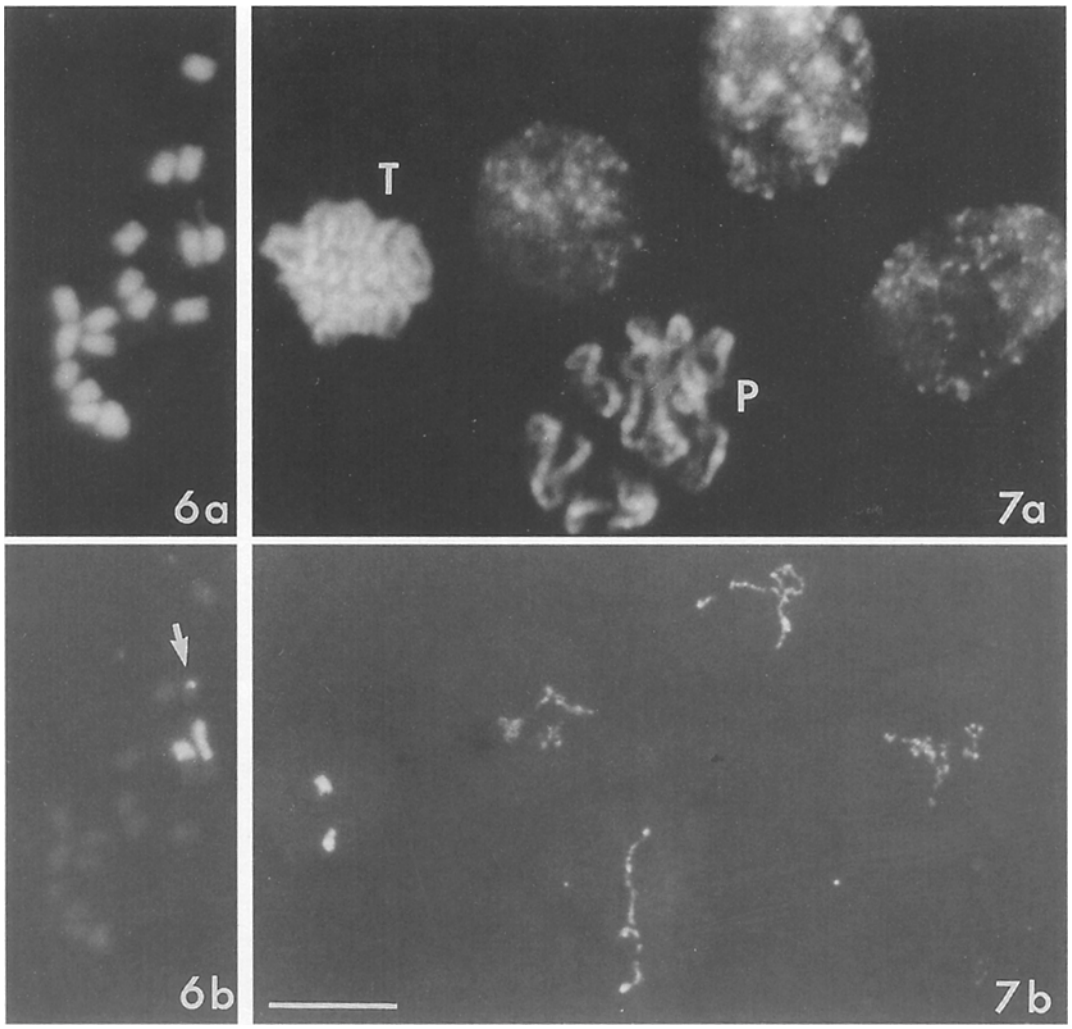

Figs. 6 and 7 Decondensation of 18S-5.8S-25S rDNA loci at metaphase and interphase, telophase (*T*) and prophase (*P*). **6 a, 7 a**. Bright fluorescence corresponds to DAPI-stained DNA. **6 b, 7 b**. Signals show the 18S-5.8S-25S rDNA loci. The *arrow* indicates the minor site. Bar 10 μm

were examined; 19 had one chromosome with an intercalary minor site, of which 13 (70%) showed hybridization on both chromatids; in five metaphases no minor site was visible and no plate showed two chromosomes with a minor hybridization signal.

At interphase, the major 18S-5.8S-25S rRNA genes showed varying degrees of decondensation. In some cases a thinner thread connected two condensed rDNA repeat clusters (Fig. 5 b), while sometimes the whole rDNA region was longer and extended forming a fine decondensed thread of rDNA chromatin through the nucleolus (Fig. 7 b). The rDNA remained decondensed during prophase and pro-metaphase (Fig. 7 b). Distinct localized rDNA signals were observed only at late metaphase, characterized by short chromosomes (Figs. 3 and 6). The two major sites were often associated (Fig. 3 b) indicating the fusion of nucleoli by the end of the preceding interphase.

Localization of 5S rRNA genes

After in-situ hybridization using digoxigenin-labelled clone pTa794 and detection with an FITC-conjugated antibody, two chromosomes showed a signal on the short arm next to the centromere (Figs. 1 b, 2 b and 3 b). Double-target in-situ hybridization (Fig. 3) with digoxigenin-labelled pTa794 and rhodamine-labelled pTa71 showed that the two 5S and the 18S-5.8S-25S rDNA major and minor sites were located on different chromosomes.

At interphase two sites which were not closely associated were observed (Figs. 1 b and 4). To determine the chromosome pair with the 5S rDNA site, the lengths of DAPI stained chromosomes from four complete metaphases were measured. The nucleolus-organizing chromosomes (pair No. 1) were identified by their lighter-stained DAPI region (see above). The remaining chromosomes were arranged by decreasing size and the chromosomes bearing the in-situ hybridization signal ranked at an average position of 3.2. Their average centromere index was calculated as 43.2%.

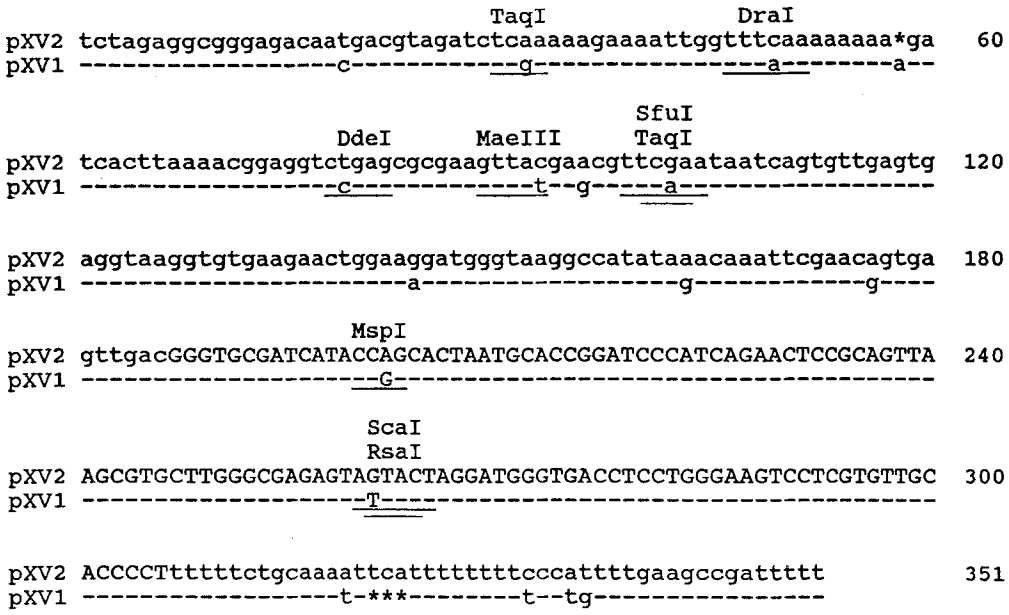

Fig. 8 Comparison of two 5S rDNA repeats of *B. vulgaris*. The intergenic spacer sequence is shown in *lower case letters* and the region coding for the 5S rRNA is shown in *upper case letters*. Sequence alterations are given. Gaps are indicated by *asterisks*. Polymorphisms in restriction enzyme recognition sites are *underlined*

Molecular structure of sugar beet 5S rDNA repeats

Restriction enzyme digestion of genomic sugar beet DNA with *Xba*I and electrophoretic separation indicated the existence of a 350 bp repeated DNA fragment and multimers thereof (data not shown). After elution and cloning, two recombinant clones (pXV1 and pXV2) were chosen for sequence analysis. A homology search within the EMBL data library revealed that both inserts contain the 5S rDNA repeat consisting of the 5S rRNA gene and the adjacent intergenic spacer. The *B. vulgaris* 5S rRNA gene is 120 bp long as determined by sequence comparison with other plant 5S rRNA genes. The coding region of the pXV2 insert shows complete identity with the sequenced 5S RNA from sugar beet (Barciszewska et al. 1987), whereas clone pXV1 differs at nucleotides 16 and 75 of the gene. An AT-rich element lies between –29 and –24 upstream of the gene; position –1 consists of a cytosine residue. The cloned 5S rDNA repeats are variants which share 94% sequence identity and have a length of 349 and 351 bp, respectively (Fig. 8). Most variation is found in the intergenic spacer (8.3%). The sequence divergence is mainly due to single nucleotide alterations. A deletion of three nucleotides was observed within the T-rich region downstream from the gene in clone pXV1. Several restriction enzyme recognition sites in both 5S rDNA repeats are affected by sequence divergence (Fig. 8).

Discussion

The data report the first application of fluorescent in-situ hybridization to chromosomes of *B. vulgaris*, an important crop species. The use of digoxigenin-labelled probes in combination with direct fluorochrome-labelled probes enabled reliable double-target in-situ hybridization, with low background and high signal strength, to the small chromosomes of cultivated beet (Figs. 1–7). Our results show that the use of fluorochrome-labelled probes for in-situ hybridization is a useful method to map repeated DNA sequences and gene families and to determine their physical relation to each other.

Localization of 18S-5.8S-25S rRNA genes

Only one pair of nucleolus-organizing chromosomes, designated chromosome 1 in most cytological studies, has been reported in mitotic cells of *B. vulgaris* (e.g., Bosemark and Bormotov 1971; de Jong and de Bock 1978). This chromosome pair is one of the largest in the complement and can be easily identified by its terminal secondary constriction (see Figs. 1 a and 2 a). Analysis at diakinesis of meiotic prophase is more sensitive than morphological analysis or silver-staining of mitotic metaphase chromosomes, since bivalents with an active nucleolus-organizing region are seen to associate with a nucleolus. Nakamura and Tsuchiya (1982) reported that at least three pairs of chromosomes appeared to have a nucleolus-organizing capacity, while Romagosa (1983, cited by Nakamura et al. 1991) found that four of the trisomic lines had extra chromosomes that associated with the nucleolus at meiotic prophase. After fluorescent in-situ hybridization (Figs. 3 b and 6 b), very strong signals were detected in all metaphase cells at or near the secondary constrictions of both chromosomes 1, while one single minor site was detected on one additional chromosome in 80% of the cells. The estimated number of rRNA genes in sugar beet is approximately 2 300 (Ingle et al. 1975). Most of the rDNA repeats are presumably localized on chromosome 1, and the very strong in-situ hybridization signal supports this. The signal strength of the single minor site in many cells suggests a copy number of approximately 20 after comparison with

the signal strength of barley minor sites (Leitch and Heslop-Harrison 1992). In-situ hybridization is often able to reveal extra rDNA sites that were not previously known, or were known only from meiotic association.

It is unlikely that there are additional rDNA loci in the cultivar Rosamona that have not been detected by our in-situ hybridization method. As beet is largely outbreeding, with a complex, multi-locus self-incompatibility system (Larsen 1977), once a polymorphism has arisen in a seed-production stock, it may be maintained. Our results do not exclude the possibility that the genome of *B. vulgaris* contains additional sites that are homologous to intergenic spacer sequences (IGS) only. Such sites would not be detected by a heterologous wheat rDNA probe where the homology to beet sequences is limited largely to the coding sequences. Rearrangements of IGS and variability in copy number and size of the 18S-5.8S-25S rRNA genes are often observed in plants (Flavell 1986; Rogers and Bendich 1987). In wheat, long-term cell suspension culture lines can loose rRNA genes and sites over a few years (Leitch et al. 1993), so perhaps rDNA sites can be lost (or presumably gained) over short-time periods, and it is likely that the varieties studied by Nakamura and Tsuchiya (1982) and Romagosa (1983, cited by Nakamura et al. 1991) had more sites than the cultivar Rosamona analyzed by us.

Interphase rDNA condensation

When the rDNA is being actively transcribed during interphase, it forms a nucleolus within which it decondenses. The active, decondensed chromatin may be barely visible in the light microscope, while dispersed, small sites, of inactive condensed chromatin are present within the nucleolus as punctuate hybridization sites (Leitch et al. 1992). Larger, unexpressed and condensed sites of rDNA are often found adjacent to the nucleolus or away from it. In cultivated beet, both major rDNA sites are expressed and may form two nucleoli or fuse to form one which often shows two individual threads of hybridization sites (Figs. 5 b and 7 b). These threads varied considerably in length and fell into two groups: (1) most of the rDNA was extended and formed long strands which might form loops but in most cases exited the nucleolus at the opposite end to the entering point and (2) shorter threads connected larger blocks of perinucleolar unexpressed sites indicating that the rDNA units in the middle of the site were expressed. The latter pattern contrasts to that seen in wheat and rye, where the distal units are expressed and one large block of unexpressed rDNA is adjacent to the proximal end of the nucleolus (Leitch et al. 1992). In general, interphase nuclei with pronounced DAPI chromocentres showed more condensed rDNA sites whereas long rDNA threads corresponded to diffusely DAPI-stained nuclei (compare Figs. 5 b and 7 b). The two different decondensation patterns could reflect different stages during the cell cycle as prophase nuclei showed long threads and telophase nuclei had condensed rDNA loci (Fig. 7 b). Alternatively, they could represent different classes of overall cell activity or proliferation.

Localization of 5S rRNA genes

Using in-situ hybridization, we identified one pair of sites of 5S RNA genes in cultivated beet, near the centromere on the short arm of one of the largest chromosomes in the complement. The centromere index of this chromosome falls into the range of centromere indices described for chromosomes 2, 3 or 4 of haploid *B. vulgaris* lines (de Jong and de Bock 1978). However, as morphological characters (length and centromere index) are not able to distinguish reliably between the very equally sized cultivated beet chromosomes (e.g., Bosemark and Bormotov 1971; de Jong and de Bock 1978; Nakamura et al. 1991), we suggest using the presence of the 5S gene cluster to identify this chromosome pair.

A centromeric location of 5S genes has also been reported for tomato (Lapitan et al. 1991), while in rye, wheat, barley and pea the 5S rDNA sites are intercalary (Ellis et al. 1988; Mukai et al. 1990; Leitch and Heslop-Harrison 1993). The 5S rRNA genes are not associated with the 18S-5.8S-25S rRNA genes in cultivated beet, as demonstrated by double-target in-situ hybridization using both probes which localize onto different chromosomes (Fig. 3b). For the in-situ hybridization experiments shown here (Figs. 1–4), we have used a clone containing the 5S rDNA sequence from *T. aestivum*. The cross hybridization to cultivated beet demonstrates the high homology between the 5S rRNA genes which have been shown to be very conserved between different species (for review see Specht et al. 1990). An increased strength of the in-situ hybridization signal was observed using the entire sugar beet 5S rDNA repeat (clone pXV1) as a probe. However, no extra sites could be detected, confirming the results achieved with the wheat probe pTa 794.

Structure and variation of 5S rDNA repeats

In higher eukaryotes, the 5S rRNA genes are arranged in long tandem arrays of 200–500 bp repeat units containing the coding sequence of about 120 bp and conserved sequence motifs which have been postulated to have a regulatory function. An AT-rich element similar to the sequence required for the transcription initiation of the 5S rRNA genes in *Neurospora* (Selker et al. 1986), and found upstream of many other plant 5S rRNA genes (Hemleben and Werts 1988; Venkateswarlu et al. 1991), is localized between –29 and –24 upstream of the sugar beet 5S rRNA gene. The C residue at –1 supports the proposed cytosine conservation at this position (Venkateswarlu et al. 1991). Clusters of T residues found downstream from the gene are assumed to be transcription termination signals (Hemleben and Werts 1988).

Recently, an RFLP-based map (Pillen et al. 1992) and the linkage of molecular markers to virus resistance genes

(Barzen et al. 1992) have been reported. The localization of 5S rRNA genes on RFLP maps, although not yet available, will allow the physical chromosome organization to be related to the genetic map, and trisomic lines (Butterfass 1963) can be used to identify the particular chromosomes. The observed variation of restriction enzyme sites within the 5S rDNA repeats could result in polymorphic restriction fragments after digestion and be valuable for RFLP mapping studies. *Taq*I, *Mae*III and *Sfu*I have multiple sites and hence should give single-digest polymorphisms. The use of 5S rDNA repeats or spacer sequences as RFLP probes for linkage analysis was described in tomato (Lapitan et al. 1991). Hypervariability of 5S ribosomal gene clusters was observed after pulsed-field-gel electrophoresis of different wheat accessions and the polymorphisms observed were proposed to serve as Mendelian markers (Röder et al. 1992).

The molecular analysis of *B. vulgaris* revealed that the genome of this crop species contains a high number of repetitive sequences (Schmidt et al. 1991; Schmidt and Heslop-Harrison 1993) and microsatellite repeats (Schmidt et al. 1993). The chromosomal localization and distribution of major classes of repeated DNA sequences is currently in progress and we aim to develop an integrated molecular and cytological model of the *B. vulgaris* genome. Fluorescent in-situ hybridization opens up new ways for detailed karyotype analysis, by providing chromosomal markers, for mapping DNA sequences and genes to chromosomes, for correlating linkage groups of genetic and RFLP maps to chromosomes, and for the positive identification of alien translocation lines that are used to transfer resistance genes from wild to cultivated beet.

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References

Barciszewska, MZ, Mashkova, TD, Kisselev, LL, Barciszewski, J (1986) The nucleotide sequence of 5S ribosomal genes from two plants: rape and white beet. Nucleic Acids Res 15:363

Barzen E, Mechelke W, Ritter E, Seitzer JF, Salamini F (1992) RFLP markers for sugar beet breeding: chromosomal linkage maps and location of major genes for rhizomania resistance, monogermy and hypocotyl colour. Plant J 2:601–611

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Phil Trans R Soc Lond B 274: 227–274

Bosemark NO, Bormotov VE (1971) Chromosome morphology in a homozygous line of sugar beet. Hereditas 69:205–212

Butterfass, T (1963) Die Chloroplastenzahlen in verschiedenartigen Zellen trisomer Zuckerrüben (*Beta vulgaris* L.). Z Bot 52:46–77

de Jong H (1981) Investigation into chromosome morphology of sugar beet and related wild species. PhD thesis, University of Amsterdam

de Jong H, de Bock TSM (1978) Use of haploids of *Beta vulgaris* L. for the study of orcein- and Giemsa-stained chromosomes. Euphytica 27:41–47

Ellis THN, Lee D, Thomas CM, Simpson PR, Cleary WG, Newman MA, Burcham KWG (1988) 5S rRNA genes in *Pisum*: sequence, long range and chromosomal organization. Mol Gen Genet 214:333–342

Flavell RB (1986) The structure and control of expression of ribosomal RNA genes. Oxford Surveys Plant Mol Cell Biol 3:251–274

Gerlach WL, Bedbrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res 7:1869–1885

Gerlach WL, Dyer TA (1980) Sequence organization of the repeating units in the nucleus of wheat which contain 5S rRNA genes. Nucleic Acids Res 8:4851–4855

Hemleben V, Werts D (1988) Sequence organization and putative regulatory elements in the 5S rRNA genes of two higher plants (*Vigna radiata* and *Matthiola incana*). Gene 62: 165–169

Heslop-Harrison JS, Schwarzacher T, Anamthawat-Jónsson K, Leitch AR, Shi M, Leitch IJ (1991) *In-situ* hybridization with automated chromosome denaturation. Technique 3:109–116

Ingle J, Timmis JN, Sinclair J (1975) The relationship between satellite desoxyribonucleic acid, ribosomal ribonucleic acid redundancy, and genome size in plants. Plant Physiol 55:496–501

Lapitan NLV, Ganal MW, Tanksley SD (1991) Organization of the 5S ribosomal RNA genes in the genome of tomato. Genome 34:509–514

Larsen K (1977) Self-incompatibility in *Beta vulgaris* L. 1. Four gametophytic complementary S-loci in sugar beet. Hereditas 85:227–248

Lehfer H, Busch W, Martin R, Herrmann RG (1993) Localization of the B-hordein locus on barley chromosomes using fluorescence in-situ hybridization. Chromosoma 102:428–432

Leitch IJ, Heslop-Harrison JS (1992) Physical mapping of the 18S-5.8S-26S rRNA genes in barley by *situ* hybridization. Genome 35:1013–1018

Leitch IJ, Heslop-Harrison JS (1993) Physical mapping of four sites of 5S ribosomal DNA sequences and one site of the α-amylase 2 gene in barley (*Hordeum vulgare*). Genome 36:517–523

Leitch AR, Mosgöller W, Shi M, Heslop-Harrison JS (1992) Different pattern of rDNA organization at interphase in nuclei of wheat and rye. J Cell Sci 101:751–757

Leitch AR, Schwarzacher T, Wang ML, Leitch IJ, Surlan-Momirovich G, Moore G, Heslop-Harrison JS (1993) Molecular cytogenetic analysis of repeated sequences in a long term wheat suspension culture. Plant Cell Tissue Org Cult 33:287–296

Maluszynska J, Heslop-Harrison JS (1991) Localization of tandemly-repeated DNA sequences in *Arabidopsis thaliana*. Plant J 1:159–166

Maluszynska J, Heslop-Harrison JS (1993) Physical mapping of rDNA loci in *Brassica* species. Genome 36:774–781

Mukai Y, Endo TR, Gill BS (1990) Physical mapping of the 5S rRNA multigene family in common wheat. J Heredity 81:290–295

Nakamura C, Tsuchiya T (1982) Pachytene chromosome morphology in diploid sugar beet, *Beta vulgaris*. Z Pflanzenzüchtg 89:229–244

Nakamura C, Skaracis GN, Romagosa I (1991) Cytogenetics and breeding in sugar beet. In: Tsuchiya T, Gupta PK (eds) Chromosome engineering in plants: genetics, breeding, evolution. Elsevier, Amsterdam Oxford New York Tokyo, pp 295–314

Pillen K, Steinrücken G, Wricke G, Herrmann RG, Jung C (1992) A linkage map of sugar beet (*Beta vulgaris* L.). Theor Appl Genet 84:129–135

Röder MS, Sorrells ME, Tanksley SD (1992) 5S ribosomal gene clusters in wheat: pulsed-field gel electrophoresis reveals a high degree of polymorphism. Mol Gen Genet 232:215–220

Rogers SO, Bendich AJ (1987) Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. Plant Mol Biol 9:509–520

Schmidt T, Heslop-Harrison JS (1993) Variability and evolution of highly-repeated DNA sequences in the genus *Beta*. Genome 36: 1074–1079

Schmidt T, Jung C, Metzlaff M (1991) Distribution and evolution of two satellite DNAs in the genus *Beta*. Theor Appl Genet 82:793–799

Schmidt T, Boblenz K, Metzlaff M, Kaemmer D, Weising, K, Kahl G (1993) DNA fingerprinting in sugar beet (*Beta vulgaris*) – iden-

tification of double-haploid breeding lines. Theor Appl Genet 85:653–657

Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989) *In-situ* localization of parental genomes in a wide hybrid. Ann Bot 64: 315–324

Schwarzacher T, Anamthawat-Jónsson K, Harrison GE, Islam AKMR, Jia JZ, King IP, Leitch AR, Miller TE, Reader SM, Rogers WJ, Shi M, Heslop-Harrison JS (1992) Genomic in-situ hybridization to identify alien chromosomes and chromosome segments in wheat. Theor Appl Genet 84: 778–786

Selker, EU, Morzycha-Wroblewska, E, Steven, JN, Metzenberg, RL (1986) An upstream signal is required for in-vitro transcription of *Neurospora* 5S RNA genes. Mol Gen Genet 205:189–192

Specht T, Wolters J, Erdmann VA (1990) Compilation of 5S rRNA and 5S rRNA gene sequences. Nucleic Acids Res (suppl.) 18:2215–2230

Venkateswarlu, K, Lee, SW, Nazar,RN (1991) Conserved upstream sequence elements in plant 5S ribosomal RNA-encoding genes. Gene 105:249–253