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A comparison of sequence resolution on plant chromosomes: PRINS versus FISH

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Abstract The resolution of the chromosomal positions of six high- and one low-copy sequences by oligonucleotide-primed in situ (PRINS) labelling was compared with corresponding data obtained after fluorescent in situ hybridization (FISH) on field-bean and barley chromosomes. While PRINS proved to be suitable for the rapid detection of high-copy tandem repeats at the same loci as those revealed by FISH, no clear PRINS signal was obtained for the low-copy family of vicilin genes at their locus on field-bean chromosome II. This indicates that localization of short target sequences by primer extension via *Taq* polymerase in situ does not yet provide a resolution equal, or superior, to FISH on plant chromosomes. Therefore, the use of a cocktail of chromosome-specific single-copy sequences as primers for PRINS is no alternative for the not as yet feasible chromosome painting in plants.

Key words Plant chromosomes · Primed in situ DNA labelling · Fluorescent in situ hybridization · Repetitive sequences

Introduction

*PR*imed *IN S*itu DNA labelling (PRINS), first described by Koch et al. (1989), is regarded as an alternative to in situ hybridization for the detection of nucleic acid sequences in chromosome and tissue preparations. It involves the annealing and extension by *Taq* polymerase of primers homologous to the target sequences. The extended sequences are labelled by the incorporation of one or more fluorochrome-, hapten- or enzyme-conjugated nucleotides. This allows physical sequence localization either directly or after additional detection procedures.

As compared to in situ hybridization the PRINS technique has several advantages: short oligonucleotide primers can be designed and synthesized with a minimum of sequence information; they hybridize to homologous targets within densely stuctured chromatin both easier and faster than the larger probes usually used for ISH; there is no need for probe labelling; primers may be extended even into flanking dispersed repetitive sequences (in less than 30 min) and thus strengthen the signal for a short unique sequence as compared to FISH with a specific probe.

In human cytogenetics, PRINS has been used for the mapping of repetitive and low-copy sequences (Gosden et al. 1991; Gosden and Lawson 1994), for chromosome identification (Gosden 1996), for the detection of aneuploidy in sperm cells (Pellestor et al. 1996), for specifying chromosome rearrangements by a combination of PRINS labelling of chromosome-specific alphoid sequences and chromosome painting (Hindkjaer et al. 1995), for PRINS on extended DNA fibers (Shibasaki and Gosden 1997), and as multi-PRINS for the sequential detection of several loci on the same chromosomes (Volpi and Baldini 1993). Even the localization of a single-copy gene has been reported (Cinti et al. 1993), although this is not yet a routine application. To improve the resolution for sequence detection, a cycling PRINS (5–10 denaturation-extension cycles) technique was developed by Terkelsen et al. (1993).

On the chromosomes of legumes (*Vicia faba*, *Pisum sativum*, *Trifolium repens*), cereals (*Hordeum vulgare*, *Avena sativa*, *Triticale*), and grasses (*Lolium multiflorum* × *Festuca arundinacea*) tandem repetitive sequences (e.g. $TTTAGGG_n$, *Fok*I-elements, sequences

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encoding 18/25S and 5S rRNA genes) have been localized by PRINS (Abbo et al. 1993 a; Macas et al. 1995; Pich et al. 1995; Thomas et al. 1996; Kubaláková et al. 1997).

Up to now, the reliable and reproducible detection of single-copy sequences below 10 kb in large plant genomes has proven difficult by FISH (Jiang and Gill 1994; Fuchs and Schubert 1995; Fuchs et al. 1998). Probes derived from genomic clones with large inserts frequently yield FISH signals over the entire chromosome complement due to dispersed repeats which flank the unique sequence of interest (Fuchs et al. 1994 a). Also chromosome painting (or chromosome in situ suppression hybridization, Lichter et al. 1988) is not yet possible for plant chromosomes due to the high complexity of the investigated genomes (Fuchs et al. 1996). Here we compare the sensitivity of PRINS and FISH for the detection of repetitive and low-copy sequences on plant chromosomes in order to test the potential of PRINS for the localization of short unique sequences and as an alternative to FISH for chromosome painting in plants.

Materials and methods

Chromosome preparation

Using a reconstructed *V. faba* karyotype with distinguishable chromosome pairs (ACB, Döbel et al. 1978) suspensions of metaphase chromosomes were obtained from synchronized root tips (~ 2 cm) of young seedlings and dropped on slides as described by Schubert et al. (1993). From barley root-tip meristems squash preparations were made according to Schubert et al. (1998). Slides were used immediately, stored dry at room temperature for several weeks or in glycerol at 4°C for up to 6 months.

Primed in situ labelling (PRINS)

Prior to PRINS, slides were washed three times in 2 × SSC for 5 min at room temperature followed by an RNase-treatment (50 µg/ml 2 × SSC for 40 min at 37°C). Subsequently a fill-in-reaction was carried out to reduce background signals caused by nicks within the chromosomal DNA which may induce polymerase activity at sites of free 3′OH-ends. For this purpose the slides were first washed in 2 × SSC and equilibrated in *Taq*-polymerase buffer (10 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$) for 5 min. Then, 20 µl of the reaction mixture containing 1 × PCR buffer (Boehringer) with 1.5 mM $MgCl_2$, 100 µM of each dATP, dCTP, dGTP, 100 µM of 2′,3′-dideoxy (dd)TTP and 2 U of *Taq*-DNA polymerase (Boehringer) were dropped onto each slide, covered with a coverslip and sealed with Fixogum rubber cement. Slides were heated to 93°C for 90 s followed by 72°C for 20 min in a wet chamber (Zytotherm, Schutron). Before labelling, the chromosomes were washed and equilibrated again. Labelling was done in a 25 µl reaction volume consisting of 1 × PCR buffer, 1.5 mM $MgCl_2$, 100 µM of each dATP, dCTP, dGTP, 75 µM of dTTP and 25 µM of digoxygenin-11-dUTP or fluorescein-12-dUTP (Boehringer), 4 µM of each of the corresponding oligonucleotide primers and 2.5 U of *Taq*-DNA polymerase. After sealing the coverslip, chromosomes were denatured for 2–3 min at 93°C. Primers were annealed at 55–60°C for 10 min and extended at 72°C for 40 min.

The reaction was stopped by washing slides twice in 4 × SSC, 0.1% Tween 20 for 5 min at 42°C. Preparations labelled with FITC-dUTP were counterstained with propidium iodide/4′,6′-diamidino-2-phenylindole dihydrochloride (PI/DAPI, 1 µg/ml each in antifade) and inspected immediately. Incorporated Dig-dUTP was detected with anti-Dig-FITC-Fab fragments from sheep (2 µg/ml, Boehringer): samples were incubated in blocking solution (4 × SSC, 0.1% Tween 20, 3% BSA) at 37°C for 30 min. Subsequently, Fab-fragments were applied in detection buffer (4 × SSC, 0.1% Tween 20, 1% BSA) for 50 min at 37°C.

Unspecifically bound conjugates were removed by washing slides in wash buffer three times for 5 min at 42°C. After counterstaining (see above) slides were inspected using a Zeiss epifluorescence microscope.

Several oligonucleotides were used separately, or in various combinations, as primers for PRINS. Their sequences and origins are given in Table 1.

Table 1 Sequence and origin of primers used for chromosomal localization by PRINS of high- and low-copy repeats

Number	Sequence		Reference
154-26 155-27	*V. faba Fok*I repeat	5′-GCCTTGTCATTATGGAAGGTAGTCTG-3′ 5′-CCATCCATTGGAGTAACAAAAACTTCG-3′	Kato et al. (1984)
UP46 UP47	5S rRNA gene of *G. max*	5′-GTGCGATCATACCAGC(A/G)(G/T)TAATGCACCGG-3′ 5′-GAGGTGCAACACGAGGACTTCCCAGGAGG-3′	Gottlob-McHugh et al. (1990)
JF07 JF08 UP07 UP08	Large spacer of *V. faba* rDNA	5′-CGAAACGCTACGAAACTCCTTGG-3′ 5′-TCTACTTTCAATTTCACGACCGG-3′ 5′-CCGACACGCGAAAAGCCGAAAAACATG-3′ 5′-CAAGGGGCGGAAAGCAAGCCAACG-3′	Kato et al. (1985)
159 167	*Arabidopsis*-type telomere repeat	5′-$(TTTAGGG)_7$-3′ 5′-$(AAATCCC)_7$-3′	Richards and Ausubel (1988)
AB01 AB02	pVf7 repeat sequence of *V. faba*	5′-ACTTTGGGGACACTCTCAATGTA-3′ 5′-ACAAGCCCTCAGTTTATTTTGAC-3′	Maggini et al. (1991)
19-30 20-32 150-30 151-24	Vicilin gene of *V. faba*	5′-GGCAGCTACCACATTGAAAGATTCATTTCC-3′ 5′-CCAGATAAGATGGACGGTTGGTTTTGATTACC-3′ 5′-GGAAGGTAAGGCTTTCATGAGTTCAGTGGC-3′ 5′-GAAGTTCAAGTACCGAGAACCGAG-3′	Weschke et al. (1988)
2-21 118-99	HvT01 subtelomeric repeat of H. *vulgare*	5′-CGAAACTCGCATTTTGGCC-3′ 5′-AGAGTTCCCGTAACCGGCCC-3′	Belostotsky and Ananiev (1990)

Results

The chromosomal localization of five high-copy sequences and one low-copy sequence of *V. faba* and one high-copy sequence of *H. vulgare* was investigated by PRINS in comparison with FISH.

*Fok*I-elements of *V. faba*

The *Fok*I-element units consist of 59 bp, are conserved within the genome and arranged as tandem repeats (Kato et al. 1984). Their copy number in *V. faba* was determined to be 5×10^6 to 5×10^7 per 2C (Kato et al. 1984) corresponding to approximately 3×10^5 to 3×10^6 kb ($\cong 0.3$ to 3 pg) or about 1–10% of the nuclear genome.

Pich et al. (1995) obtained bright fluorescent signals after PRINS using a single primer (154-26, see Table 1). In the present work a pair of primers (154-26, 155-27), designed to span the entire *Fok*I repeat, was employed. When Dig-dUTP was used for labelling, the indirect detection yielded strong signals on all chromosomes with the exception of chromosome III of karyotype ACB. Also minor dot-like signals on chromosomes I, II, IV and VI appeared frequently (Fig. 1 b). This confirmed the localization of *Fok*I sequences by fluorescence in situ hybridization (Fuchs et al. 1994 a). The fact that minor loci close to the centromeres of chromosomes I and VI were not observed by Fuchs et al. (1994 a) but appeared later occasionally on other chromosome preparations indicates an inter-individual polymorphism of minor *Fok*I-element positions. This is supported by rare minor loci in chromosome regions which, when microdissected from individuals without the corresponding FISH signals, do not yield PCR amplification of *Fok*I elements with *Fok*I-element-specific primers (Fuchs et al., unpublished).

The highly repetitious character of the *Fok*I sequence at the main loci allowed us to visualize these positions even by incorporating fluorochrome-labelled nucleotides (FITC-dUTP). This approach was characterized by an optimal signal-to-noise ratio but at a lower sensitivity since the minor loci did not appear as clear signals (Fig. 1 a).

5S rRNA genes

The 5S rRNA genes are repetitive sequences which occur in all eukaryotic genomes. In many plant species they are organized as tandem repeats each comprising a highly conserved 120-bp coding region and a non-coding spacer of species-specific length and nucleotide sequence. The length of the basic repeats of *Glycine max* and *Phaseolus vulgaris* is 330 and 389 bp, respectively (Gottlob-McHugh et al. 1990). For *Vigna radiata* a repeat number of 4300 was estimated (Hemleben and Werts 1988). Therefore it seems reasonable to assume that the 5S rRNA genes of *V. faba* may comprise about 10^3 kb.

The primers for PRINS (Table 1) were designed according to the coding region of *G. max*. The basic PCR product represents 117 bp starting at position + 3.

Indirect, as well as direct, PRINS labelling resulted in dot-like signals at two loci on the satellite of the long arm of chromosome III of karyotype ACB (Fig. 3). This agrees with the results obtained after radioactive in situ hybridization (Knälmann and Burger 1977; Schubert et al. 1978) and FISH experiments (Fuchs et al. 1998). With indirect labelling weaker signals occasionally appeared at the most prominent heterochromatic positions (Fig. 3 b,c) characterized by strong Giemsa bands (Fuchs et al. 1998).

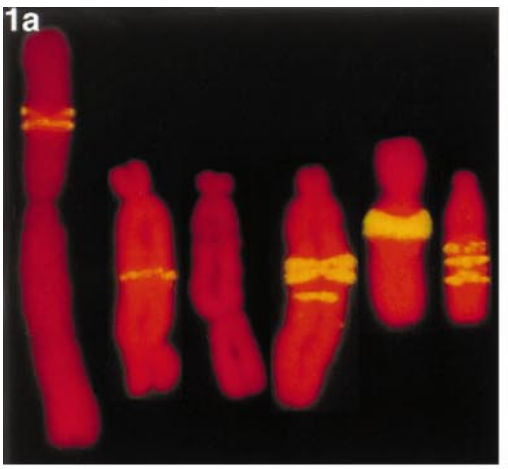
Nucleolus organizing regions

Tandem repeats of the genes encoding 5.8, 18 and 25S rRNA, as well as transcribed and non-transcribed spacers, are contained in the nucleolus-organizing regions (NORs). In *V. faba* a single NOR is easily visible as a secondary constriction on the long arm of chromosome III of karyotype ACB. Due to the variable length of the non-transcibed spacer the total size of rDNA repeats may vary to a large extent even within individuals. The size of the *V. faba* rDNA repeat units was determined to be 8 to 13 kb (Yakura and Tanifuji 1983; Kato et al. 1985, 1990) with a copy number of about 9500 per cell. Thus, the size of a NOR in *V. faba* may span $4–6 \times 10^4$ kb (for a review see Hemleben et al. 1988).

For PRINS labelling of the *V. faba* NOR, two pairs of oligonucleotides (Table 1) derived from the large spacer with a distance of 151 and 313 bp, respectively, were used as primers. In addition a 3.7-kb *Eco*RI fragment cloned in pBR325 (VER17, Yakura and Tanifuji 1983) was cleaved with *Hpa*II and *Taq*I and the digestion-products were used for priming. As with in situ hybridization experiments (for a review see Fuchs et al. 1998), only the NOR revealed a clear signal after indirect PRINS labelling using either the oligonucleotides or the restriction fragments of VER17 as primers (Fig. 4).

Telomeric sequences

$TTTAGGG_n$ is the telomeric repeat of *A. thaliana* (Richards and Ausubel 1988) and most other plant species including *V. faba* (for a recent review see Fuchs and Schubert 1996). Heptamers of this basic unit were used as primers for PRINS labelling by Dig-dUTP in the present work. Fluorescent signals were obtained at all 12 chromosome ends of *V. faba*. Additionally, two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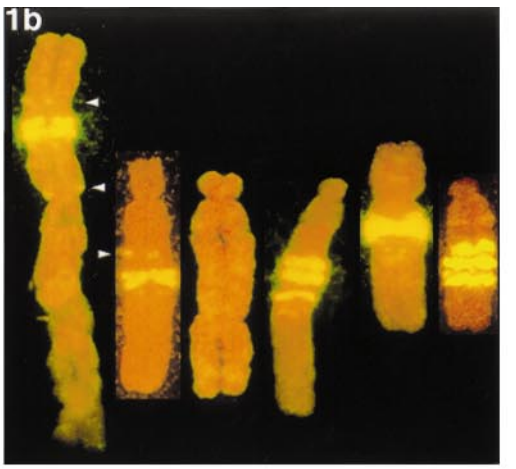
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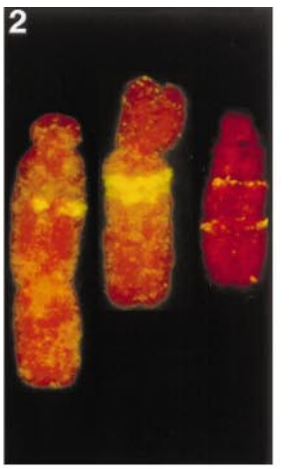
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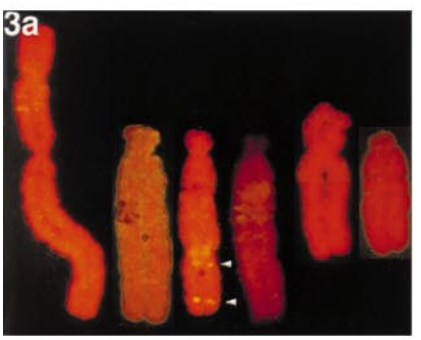
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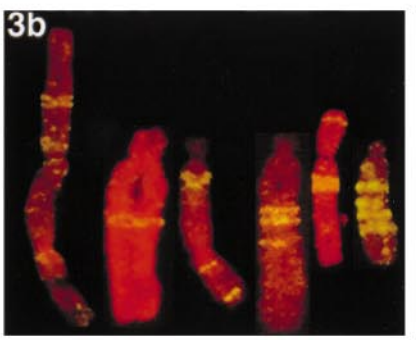
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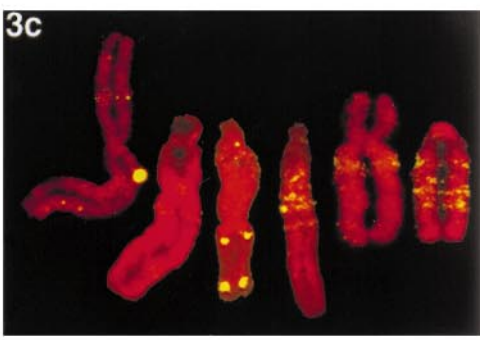
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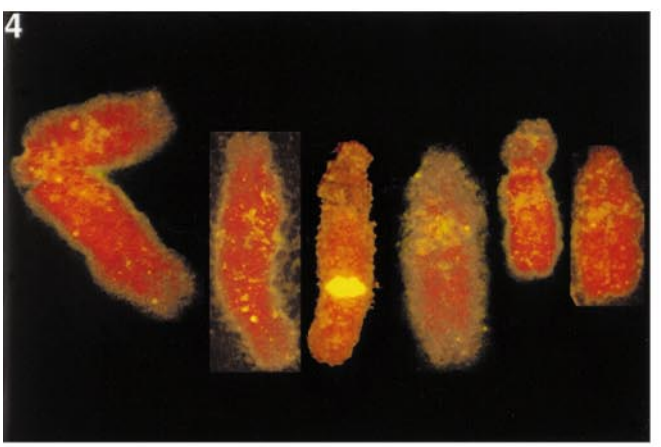
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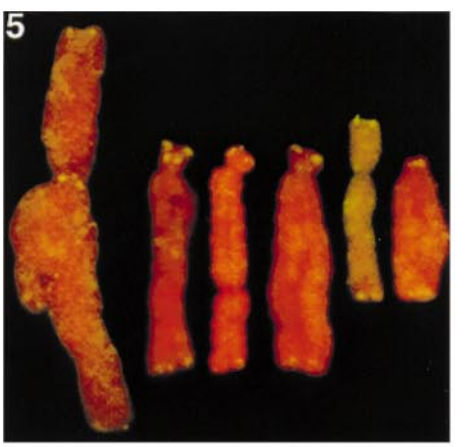
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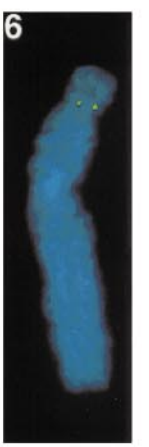
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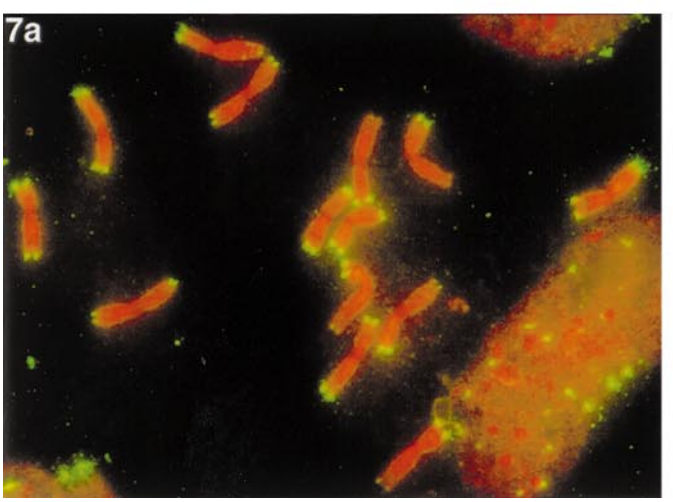
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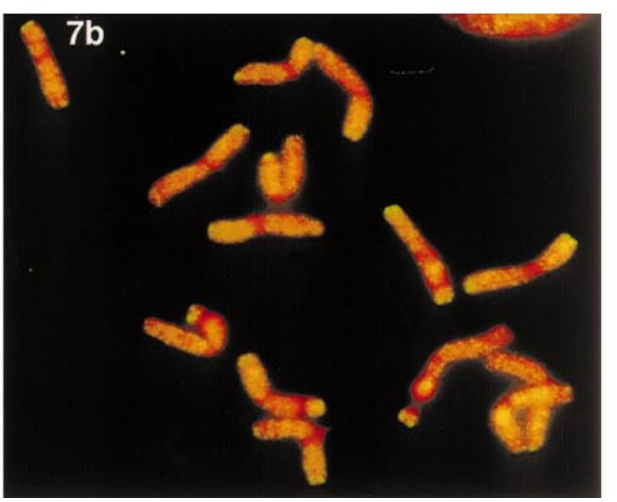
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interstitial sites were located at the centromere of chromosome I and within the short arm of chromosome II, respectively (Fig. 5). This corresponds to previous results obtained by FISH (Schubert 1992). However, not every one of the chromosomes of the sample revealed a detectable signal. Since the target size of telomeric repeats is estimated from the intensity of FISH signals to comprise at least 20 kb per locus, these experimental data may be indicative of the actual resolution limitation of the PRINS technique using Dig-labelled nucleotides.

pVf7 repeats

pVf7 represents a 335-bp repeat related to a sequence of the intergenic spacer of the polycistronic rDNA unit of *V. faba* (Maggini et al. 1991). After incorporation of Dig-dUTP, PRINS with primers spanning 320 bp of the repeat unit (Table 1) yielded the same five signals on chromosomes III, IV, V and VI of the *V. faba* ACB karyotype as were found after FISH with digoxygenin-labelled PCR products obtained with the same primers from genomic DNA (Fig. 2, Fuchs et al. 1998).

Vicilin genes

Vicilin genes encode a major seed-storage protein of 50 kDa of *V. faba*. A single such gene contains five introns and comprises 2.2 kb (Weschke et al. 1988). Vicilin genes form a small gene family of about five members at a single locus, mapped by PCR with microdissected chromosomes (Macas et al. 1993) and by FISH (Fig. 6 and Fuchs et al. 1994 b), on the short arm of chromosome II near the centromere. Since only one copy of the gene was found within genomic λ-clones containing inserts of 12–15 kb; this value can be regarded as the minimum distance between the members of this gene family.

Four different oligonucleotides were simultaneously used as primers for PRINS labelling (Table 1). Two were designed to yield a 841-bp PCR product, whereas two others were directed outwards to label regions beyond the start and stop codons.

With these primers no clear and reproducible PRINS signal could be obtained within the vicilin gene locus. Apparently, the number of the incorporated labelled nucleotides and/or the copy number of the target sequences were too small. Alternatively, it might be assumed that the single stretches of labelled DNA were too far from each other to provide a joint resolvable fluorescence signal.

Subtelomeric repeats of barley

A 118-bp tandem repeat, HvT01, of barley (Belostotsky and Ananiev 1990) comprising up to 1 Mb per locus was found to be associated with telomeric sequences (Kilian and Kleinhofs 1992; Röder et al. 1993) and was detectable by FISH at all 14 chromosome termini of barley (Fig. 7 a, Schubert et al. 1998). PRINS with primers (Table 1) spanning positions 2–21 and 118–99 of the tandem repeat yielded, after incorporation of Dig-dUTP, signals at the barley chromosome termini. However, the background was higher than after FISH with a Dig-labelled PCR product obtained with the same primers from genomic DNA of barley. Positions of weak terminal (2HL, 5HL) or subterminal (3H, 4H) FISH signals were not clearly distinguishable from the background (Fig. 7 b).

Discussion

We have confirmed (*Fok*I elements, telomeric repeats, 18/25 S rRNA genes of *V. faba*: Macas et al. 1995; Pich et al. 1995; Kubaláková et al. 1997) and extended (5S rRNA genes, pVf7 repeats of *V. faba*, HvT01 repeats of barley) the detectability of high-copy repeats by PRINS labelling of plant chromosomes. All loci were found to be identical to those revealed by FISH. The results have shown that PRINS labelling offers a suitable tool for the rapid localization of tandem repetitive sequences on plant chromosomes when primer distances are small (<1 kb) and target sequences are large (≥ 20 kb). Obviously, the extension of primer sequences by *Taq* polymerase is not yet sufficient to yield clear signals for targets, such as the vicilin genes, which are reproducibly detectable by FISH when a sequence of 2.2 kb, which is repeated five times within the genome but with a distance of >12 kb from each other, is used as a probe. Abbo et al. (1993 b) reported the detection of the hordein-B gene cluster (10–15 genes of 873 bp) on barley chromosome 1HS by weak PRINS signals using two primers with a distance of about 600 bp. However, these authors provided no data as to

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Fig. 1 A haploid chromosome complement of the field-bean karyotype ACB after **a** direct (FITC-dUTP) and **b** indirect (Dig-dUTP) PRINS-labelling of *Fok*I element loci

Fig. 2 Indirect PRINS labelling of pVf7 loci on chromosomes III (one signal), V and VI (two signals each) of the field-bean karyotype ACB

Fig. 3 Field-bean chromosomes (karyotype ACB) after PRINS labelling with 5S rRNA gene-specific primers: **a** direct labelling **b** indirect labelling without and **c** with incorporation of ddTTP prior to PRINS. The triangles in a mark the gene positions

Fig. 4 Field-bean chromosomes after indirect PRINS labelling of the NOR using the primers JF07 and JF08 (see Table 1).

Fig. 5 Indirect PRINS labelling of telomeric sequences on *V. faba* chromosomes

Fig. 6 Field-bean chromosome II after FISH with a biotinylated 4-kb *Eco*RI fragment of a genomic vicilin clone detected by streptavidin-FITC (Fuchs et al. 1994)

Fig. 7 Metaphases of barley cv 'Betzes' after detection of the subtelomeric repeat HvT01 **a** by FISH with a Dig-dUTP-labelled probe (Schubert et al. 1998) and **b** by indirect PRINS labelling

the frequency of signal appearance; therefore the reproducibility of these results remains unclear.

A major disadvantage of PRINS labelling as compared to FISH proved to be the relatively high background along the chromosomes, which is most probably due to the incorporation of labelled nucleotides by *Taq* polymerase at free 3′OH-ends. This is true, although at lower level, even after an extension reaction with unlabelled nucleotides and ligase treatment (data not shown) or after incorporation of dideoxy-TTP prior to PRINS labelling. It cannot be excluded that DNA breaks may arise anew by heating during the denaturation step. This may be the reason for the finding that cycling PRINS with several denaturation/annealing/extension cycles increases the intensity of (strong) signals but also yields a higher background (Kubaláková et al. 1997), thus preventing the detection of the weak signals expected for single-copy loci.

Another problem is the unspecific labelling that occasionally occurred (in addition to the signals at primer-specific target loci) in heterochromatic regions of *V. faba*, known to be composed of *Fok*I repeats and other repetitive sequences (Fig. 3 b,c). Possibly, in some preparations these regions contain, for unknown reasons, clusters of breaks and therefore free 3′OH-ends. Although pre-treatment for reduction of 3′OH-ends by incorporation of ddTTP usually suppresses the general background, as well as unspecific heterochromatin labelling, the remaining unspecific signals may be misleading when unknown sequence positions have to be determined.

Direct labelling by PRINS with fluorochrome-conjugated nucleotides resulted in a lower background but also in a weaker signal intensity. This application is therefore restricted to very large targets.

Altogether, our results and previous data indicate that PRINS at present does not reveal loci of short single-copy sequences with a higher efficiency than FISH, and therefore does not yet offer an alternative for painting plant chromosomes by the use of cocktails of chromosome-specific single-copy clones as primers, although PRINS proved to be useful for fast in situ detection of high-copy tandem repeats.

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References

Abbo S, Miller TE, King IP (1993 a) Primer-induced in situ hybridization to plant chromosomes. Genome 36 : 815–817

Abbo S, Dunford RP, Miller TE, Reader SM, King IP (1993 b) Primer-mediated in situ detection of the B-hordein gene cluster on barley chromosome 1H. Proc Natl Acad Sci USA 90 : 11821–11824

Belostotsky DA, Ananiev EV (1990) Characterization of relic DNA from barley genome. Theor Appl Genet 80 : 374–380

Cinti C, Santi S, Maraldi NM (1993) Localization of single-copy genes by the PRINS technique. Nucleic Acids Res 21 : 5799–5800

Döbel P, Schubert I, Rieger R (1978) Distribution of heterochromatin in a reconstructed karyotype of *Vicia faba* as identified by banding- and DNA late-replication patterns. Chromosoma 69 : 193–209

Fuchs J, Schubert I (1995) Localization of seed protein genes on metaphase chromosomes of *Vicia faba* via fluorescence in situ hybridization. Chromosome Res 3 : 94–100

Fuchs J, Schubert I (1996) *Arabidopsis*-type telomere sequences on chromosome termini of *Selaginella martensii* Spring (Pteridophyta). Biol Zentbl 115 : 260–265

Fuchs J, Pich U, Meister A, Schubert I (1994 a) Differentiation of field bean heterochromatin by in situ hybridization with a repeated *Fok*I sequence. Chromosome Res 2 : 25–28

Fuchs J, Joos S, Lichter P, Schubert I (1994 b) Localization of vicilin genes on field bean chromosome II by fluorescent in situ hybridization. J Hered 85 : 487–488

Fuchs J, Houben A, Brandes A, Schubert I (1996) Chromosome 'painting' in plants – a feasible technique? Chromosoma 104 : 315–320

Fuchs J, Strehl S, Brandes A, Schweizer D, Schubert I (1998) Molecular-cytogenetic characterization of the *Vicia faba* genome – heterochromatin differentiation, replication patterns and sequence localization. Chromosome Res 6 : 219–230

Gosden JR (1996) Identification and quantitation of human chromosomes by primed in situ synthesis. Chromosome Res 4 : 331–334

Gosden J, Lawson D (1994) Rapid chromosome identification by oligonucleotide-primed in situ DNA synthesis (PRINS). Human Mol Genet 3 : 931–936

Gosden J, Hanratty D, Starling J, Fantes J, Mitchell A, Porteous D (1991) Oligonucleotide-primed in situ DNA synthesis (PRINS): a method for chromosome mapping, banding, and investigation of sequence organization. Cytogenet Cell Genet 57 : 100–104

Gottlob-McHugh SG, Lévesque M, MacKenzie K, Olson M, Yarosh O, Johnson DA (1990) Organization of the 5 S rRNA genes in the soybean *Glycine max* (L.) Merrill and conservation of the 5S rDNA repeat structure in higher plants. Genome 33 : 486–494

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

Hemleben V, Ganal M, Gerstner J, Schiebel K, Torres RA (1988) Organization and length heterogeneity of plant ribosomal RNA genes. In: Kahl G (ed) Architecture of eukaryotic genes. VCH, Weinheim, pp 371–383

Hindkjær J, Brandt CA, Koch J, Lund TB, Kolvraa S, Bolund L (1995) Simultaneous detection of centromere-specific probes and chromosome painting libraries by a combination of primed in situ labelling and chromosome painting (PRINS-painting). Chromosome Res 3 : 41–44

Jiang J, Gill BS (1994) Non-isotopic in situ hybridization and plant genome mapping: the first 10 years. Genome 37 : 717–725

Kato A, Yakura K, Tanifuji S (1984) Sequence analysis of *Vicia faba* repeated DNA, the *Fok*I repeat element. Nucleic Acids Res 12 : 6415–6426

Kato A, Yakura K, Tanifuji S (1985) Repeated DNA sequences found in the large spacer of *Vicia faba* DNA. Biochim Biophys Acta 825 : 411–415

Kato A, Nakajima T, Yamashita J, Yakura K, Tanifuji S (1990) The structure of the large spacer region of the rDNA in *Vicia faba* and *Pisum sativum*. Plant Mol Biol 14 : 983–993

Kilian A, Kleinhofs A (1992) Cloning and mapping of telomere-associated sequences from *Hordeum vulgare* L. Mol Gen Genet 235 : 153–156

Knälmann M, Burger E-C (1977) Cytologische Lokalisation von 5S und 18/25S RNA Genorten in Mitose-Chromosomen von *Vicia faba*. Chromosoma 61 : 177–192

Koch JE, Kolvraa S, Petersen KB, Gregersen N, Bolund L (1989) Oligonucleotide-priming methods for the chromosome-specific labelling of α satellite DNA in situ. Chromosoma 98 : 259–265

Kubaláková M, Macas J, Dolezel J (1997) Mapping of repeated DNA sequences in plant chromosomes by PRINS and C-PRINS. Theor Appl Genet 94 : 758–763

Lichter P, Cremer T, Borden J, Manuelidis L, Ward DC (1988) Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. Hum Genet 80 : 224–234

Macas J, Weschke W, Bäumlein H, Pich U, Houben A, Wobus U, Schubert I (1993) Localization of vicilin genes via polymerase chain reaction on micro-isolated field bean chromosomes. Plant J 3 : 883–886

Macas J, Dolezel J, Gualberti G, Pich U, Schubert I, Lucretti S (1995) Primer-induced labelling of pea and field bean chromosomes in situ and in suspension. BioTechniques 19 : 402–408

Maggini F, Cremonini R, Zolfino C, Tucci GF, D'Ovidio R, Delre V, DePace C, Scarascia Mugnozza GT, Cionini PG (1991) Structure and chromosomal localization of DNA sequences related to ribosomal subrepeats in *Vicia faba*. Chromosoma 100 : 229–234

Pellestor F, Quennesson I, Coignet L, Girardet A, Andréo B, Charlieu JP (1996) Direct detection of disomy in human sperm by the PRINS technique. Hum Genet 97 : 21–25

Pich U, Meister A, Macas J, Dolezel J, Lucretti S, Schubert I (1995) Primed in situ labelling facilitates the flow sorting of similar sized chromosomes. Plant J 7 : 1039–1044

Richards EJ, Ausubel FM (1988) Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. Cell 53 : 127–136

Röder MS, Lapitan NLV, Sorrells ME, Tanksley SD (1993) Genetic and physical mapping of barley telomeres. Mol Gen Genet 238 : 294–303

Schubert I (1992) Telomeric polymorphism in *Vicia faba*. Biol Zentbl 111 : 164–168

Schubert I, Bäumlein H, Wobus U (1978) In situ hybridization of iodinated 5 S and 18/25 S RNA to *Vicia faba* metaphase chromosomes. Biol Zentbl 97 : 129–135

Schubert I, Dolezel J, Houben A, Scherthan H, Wanner G (1993) Refined examination of plant metaphase chromosome structure at different levels made feasible by new isolation methods. Chromosoma 102 : 96–101

Schubert I, Shi F, Fuchs J, Endo TR (1998) An efficient screening for terminal deletions and translocations of barley chromosomes added to common wheat. Plant J 14 : 489–495

Shibasaki Y, Gosden JR (1997) Oligonucleotide PRINS DNA synthesis on extended chromatin preparations. In: Gosden JR (ed) PRINS and in situ PCR protocols. Methods Mol Biol 71 : 45–51

Terkelsen C, Koch J, Kolvraa S, Hindkjr J, Pedersen S, Bolund L (1993) Repeated primed in situ labelling: formation and labeling of specific DNA sequences in chromosomes and nuclei. Cytogenet Cell Genet 63 : 235–237

Thomas HM, Williams K, Harper JA (1996) Labelling telomeres of cereals, grasses and clover by primed in situ DNA labelling. Chromosome Res 4 : 182–184

Volpi EV, Baldini A (1993) MULTIPRINS: a method for multicolour primed in situ labelling. Chromosome Res 1 : 257–260

Weschke W, Bassüner R, Hai NV, Czihal A, Bäumlein H, Wobus U (1988) The structure of a *Vicia faba* vicilin gene. Biochem Physiol Pflanz 183 : 233–242

Yakura K, Tanifuji S (1983) Molecular cloning and restriction analysis of *Eco*RI-fragments of *Vicia faba* rDNA. Plant Cell Physiol 24 : 1327–1330