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Fingerprinting plant genomes with oligonucleotide probes specific for simple repetitive DNA sequences

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Summary. Oligonucleotides hybridizing to simple repetitive DNA patterns are highly informative as probes for DNA fingerprinting in all investigated animal species, including man. Here we demonstrate the applicability of this technique in higher plants. The oligonucleotide probes $(GTG)_5$ and $(GATA)_4$ were used to investigate the differences in DNA fingerprint patterns of the following angiosperm species: *Triticum aestivum, Secale cereale, Hordeum vulgare, Beta vulgaris, Petunia hybrida, Brassica oleracea,* and *Nicotiana tabacum.* Two species, *Hordeum vulgare* as a monocot and *Beta vulgaris* as a dicot, were analyzed in more detail. Their genomes differ considerably in both amount and organization of the simple repetitive sequences $(GATA)_n$, $(GACA)_n$, $(GTG)_n$, and $(CT)_n$ due to the evolutionary distance of these two species. Furthermore, several lines and cultivars of *Beta vulgaris* and *Hordeum vulgare* can clearly be distinguished on the basis of their highly polymorphic patterns of these repetitive sequences.

Key words: DNA fingerprinting – Repetitive DNA – Genotype identification – Angiosperms

Introduction

In contrast to prokaryotes, eukaryotic genomes are characterized by the occurrence of large amounts of repetitive DNA. In the early 1970s, a new type of repetitive DNA was discovered consisting of simple short units reiterated in tandem to form repetitive nucleotide sequences of about 0.1–20 kb. These sequences were initially called 'simple sequences,' but are now often collectively referred to as "minisatellites" or as "variable number of tandem repeats" (cf. review by Jarman and Wells 1989). The simplest repetitive sequences are constituted from short (2–4 bp) motives reiterated over and over in tandem (Epplen 1988; Tautz 1989). DNA probes hybridizing to families of minisatellite DNA or to those simple tandem repeats revealed a surprisingly high degree of variability in man and various animals. First described in human DNA, the complex pattern of hybridizing DNA restriction fragments was dubbed "DNA fingerprint" (Jeffreys et al. 1985). The high variability of DNA fingerprints and their Medelian type of inheritance allow identification of individuals (genotypes) in man, other mammals and, according to more recent data, also in birds, fungi, and protozoa (Ryshkov et al. 1988; Epplen 1988; Rogstad et al. 1988; Tautz 1989). The potential of individualization on the DNA level opens up a great variety of applications, including forensic investigations, paternity testing, population and ecological genetics. Furthermore, breeders may use DNA fingerprinting to identify interesting genotypes, varieties, cultivars, and genome mutnats, or to determine the degree of variability in populations, etc.

To apply DNA fingerprinting in plant biology and plant breeding, it has to be demonstrated that these types of repetitive sequences are present in plant genomes, especially of crop plants, and that they show variability. First promising results with higher plants have been reported for rice (Dallas 1988), tomato, one gymnosperm species, and three angiosperm species (trees) (Rogstad et al. 1988), using probes that detect minisatellite sequences. Encouraging results have also been obtained with the oligonucleotides $(GATA)_4$ and $(GACA)_4$ as probes in DNA isolated from barley and chickpea (Weising et al. 1989). However, a systematic screening of plant genomes and thorough studies on the extent of variabil-

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ity within species are lacking. In the following, we demonstrate the successful use of oligonucleotide probes for fingerprinting various monocotyledonous and dicotyledonous plant species, and provide evidence for the variability of fingerprints within barley and sugar beet.

Materials and methods

Plant material

The following species and cultivars were used in our studies: *Hordeum vulgare L.* 'Salome,' 'Femina,' 'Zenit' (obtained from Dr. R. Hunold, AdL, Aschersleben), 'Haisa' (Dr. F. Scholz, AdW, Gatersleben), 'Borwina' (Dr. K. Adolf, AdL, Gülzow); *Secale cereale L.* 'Esto' and *Triticum aestivum L.* 'Chinese Spring' (Dr. G. Melz, AdL, Güstrow); *Beta vulgaris L.* var. *altissima*, cytoplasmic male-sterile and fertile plants (Prof. Dr. R. Melzer, AdL, Klein Wanzleben); *Brassica oleracea L.* 'Dithmarscher Frühling' (Dr. E. Roth, AdL, Quedlinburg); *Daucus carota sativus* 'Vitaminaja' (Dr. T. Nothnagel, AdL, Quedlinburg); *Nicotiana tabacum L.* 'Samsun' (Dr. W. Hess, HUB, Berlin); *Petunia hybrida* 'Mitchell', haploid line (Dr. F. Köhler, FU, Berlin).

Isolation of plant DNA, restriction enzyme digestion, and gel electrophoresis

DNA was isolated from leaves of individual plants using the CTAB method (Rogers and Bendich 1985). Three micrograms DNA was digested according to supplier's recommendations with the restriction enzymes *Hin*fI, *Mbo*I, *Alu*I, and *Hae*III (BRL). Samples were loaded onto 30 cm long, horizontal 0.7% agarose gels in TAE buffer (40 m*M* Tris; 12 m*M* sodium acetate; 2 m*M* EDTA, pH 8.3). Gels were run at 1 V/cm for up to 48 h and dried on a vacuum gel dryer, according to Tsao et al. (1983).

Probes and hybridizations

The oligonucleotides $(GATA)_4$, $(GACA)_4$, $(GTG)_5$, and $(CT)_8$ were synthesized on an automated DNA synthesizer (Applied Biosystem model 381 A). 5′-Endlabeling of the oligonucleotides was carried out by a T4-DNA-kinase reaction using γ-^{32}P-ATP (Amersham). Labeled probes were purified through a DE52 column (Whatman) according to Schaefer et al. (1988).

Gels were denatured in 0.5 *M* NaOH/0.15 *M* NaCl for 15 min and neutralized in 0.5 *M* TRIS-HCl (pH 8)/0.15 *M* NaCl for 30 min at ambient temperature, followed by equilibration in 6 × SSC (20 × SSC: 3 *M* NaCl, 0.3 *M* sodium citrate) prior to hybridization. After 30 min of prehybridization at the respective hybridization temperature [$(GATA)_4$: 35 °C; $(GACA)_4$ and $(CT)_8$: 43 °C; $(GTG)_5$: 45 °C], 1–2 × 10^6 cpm/ml of the probe was added to the prehybridization mix (5 × SSPE, 0.1% SDS, 10 µg sonicated and denatured *E. coli* DNA). After hybridization, gels were washed in 6 × SSC for 20 min at ambient temperature, followed by a wash at the hybridization temperature for 1–2 min. Gels were autoradiographed using XAR-5 films (Kodak) at 4 °C. Prior to reprobing, gels were incubated two to three times for 20 min in 5 m*M* EDTA (pH 7.8) at 60 °C and finally equilibrated in 6 × SSC at room temperature.

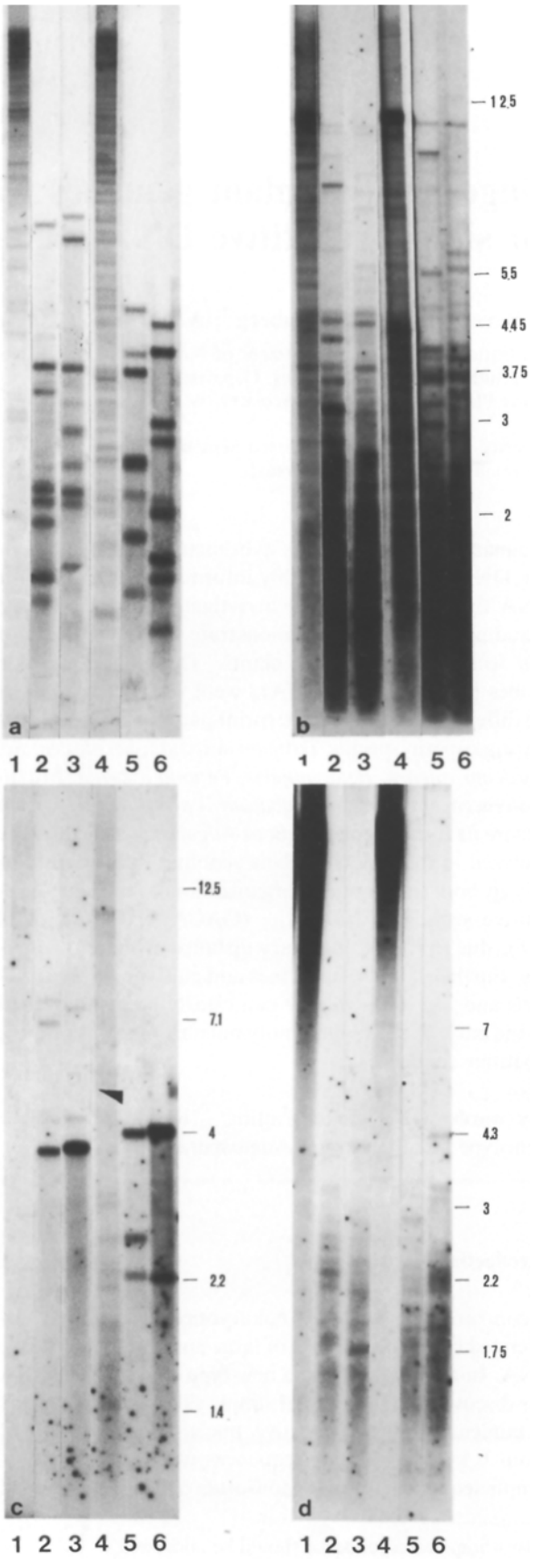

Fig. 1a–d. Hybridization patterns of successive in-gel hybridization with **a** $(GATA)_4$, **b** $(GTG)_5$, **c** $(GACA)_4$, and **d** $(CT)_8$ probes. *Lane 1: Hin*fI-digested DNA of *Hordeum vulgare* 'Salome'; *lanes 2, 3*: *Hin*fI-digested DNA of two different genotypes of *Beta vulgaris*; *lane 4: Mbo*I-digested DNA of *H.v.* 'Salome'; *lanes 5, 6*: *Mbo*I-digested DNA of those two *Beta vulgaris* genotypes

Table 1. Indicated application of the oligonucleotide probes $(GTG)_5$ and $(GATA)_4$ in higher plant species

Species	$(GTG)_5$	$(GATA)_4$
Triticum aestivum	+	+
Secale cereale	+	+
Lycopersicon esculentum	+	+
Daucus carota sativus	−	+
Brassica oleracea	−	+
Petunia hybrida	+	+
Nicotiana tabacum	+	+
Hordeum vulgare	−	+
Beta vulgaris	+	+

Results and discussion

The majority of the species tested contained simple repetitive DNA sequences (Table 1). Highly polymorphic hybridization patterns (fingerprints) were obtained unique for each species (shown for barley and sugar beet in Fig. 1). Hybridization patterns proved to be reproducible in independently isolated DNA samples from plants of the same genotype (Fig. 2, lanes 3, 4). The DNAs of two individual plants of *B. vulgaris* (sugar beet) and several cultivars of *H. vulgare* (barley) were digested to completion with four different restriction enzymes: *Hinf*I, *Mbo*I, *Alu*I, and *Hae*III. As can be seen in Fig. 1, various patterns were obtained by successive in-gel hybridizations with the probes containing simple repetitive motives. Striking differences were observed between the two species in the overall signal intensity as well as the total number of bands. The barley genome contains many $(GATA)_n$- and $(GTG)_n$-stretches on long DNA fragments without restriction enzyme recognition sites. Hybridization bands are nearly evenly distributed over a molecular weight range from 1 to 30 kb, irrespective of the enzyme used. Distinct bands were not detected with $(CT)_8$ as a probe. High-molecular-weight hybridization signals greater than 7–8 kb were detected, but could not be resolved into unique fragments. Long (GACA)n blocks were completely lacking. Hybridization signals differed dramatically in sugar beet: the patterns obtained with all four enzymes are characterized by only a few bands in the molecular range up to 10 kb in length. Significant differences were oserved in occurrence, quantity, and genomic arragement of particular simple repetitive sequences in the two crop species. In contrast to the pronounced differences between the two genotypes of sugar beet, a high number of common bands was observed in several cultivars of barley (Fig. 2). This may be interpreted as resulting from the close relationship between the cultivars studied or from a generally low degree of variability within this species.

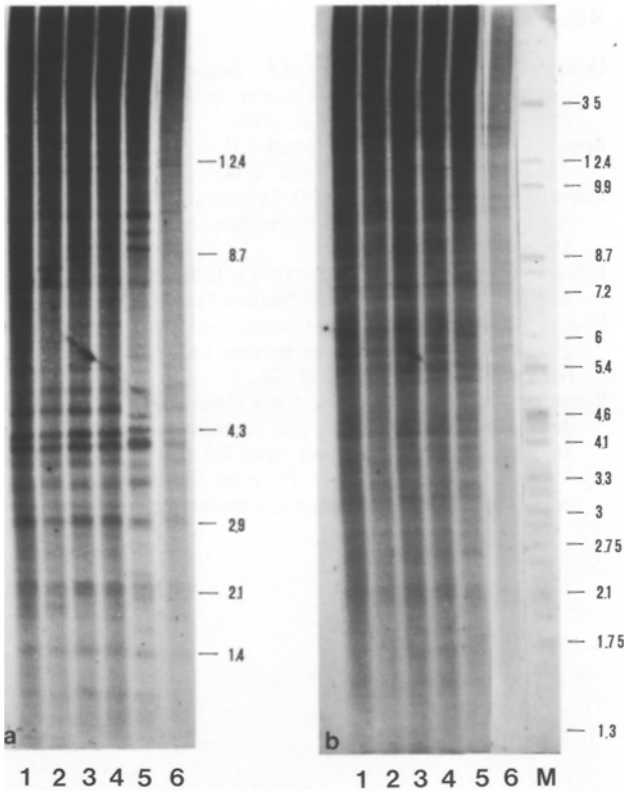

Fig. 2a, b. Successive hybridizations of *Hinf*I-digested DNA from several barley cultivars with **a** $(GATA)_4$ and **b** $(GTG)_5$ probes. *Lane 1: H.v.* 'Salome'; lane 2: *H.v.* 'Zenit'; *lanes 3, 4: H.v.* 'Femina'; *lanes 5: H.v.* 'Borwina'; *lane 6: H.v.* 'Haisa.' *M:* human DNA digested by *Hinf*I was used as standard marker for size determinations (size in all figures are given in kilobases)

In order to investigate the possibility of individualizing hitherto untested plant species, as first probe we now use routinely $(GATA)_4$, followed sometimes by $(GTG)_5$ or other probes if more information is needed. Among the species investigated so far are wheat, rye, barley, carrot, tobacco, beets, petunia, tomato, and cabbage.

DNA fingerprinting with oligonucleotides specific for simple repeats should thus be a powerful tool in breeding research. The breeding history of various lines or cultivars of a particular species may be traced; genotypes, cultivars, and varieties may be identified, as well as addition lines and chromosomal aneuploids. Furthermore, this technique should be applicable to questions in population genetics, ecology, mutation research, identification of somatic hybrids, etc. An efficient, non-radioactive hybridization procedure using oligonucleotide probes has been elaborated (Zischler et al. 1989).

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