OLIGONUCLEOTIDE FINGERPRINTING IN ANGIOSPERMS

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Simple quadruplet repeats such as $(GATA)_n$ and $(GACA)_n$ represent major constituents of the repetitive DNA in most animal genomes investigated so far (1). These sequences are thought to originate from unequal recombination and/or slipped strand mispairing and may have arisen independently in several distant taxonomic groups (2). Their functional significance, if any, is far from being clear. $(GATA)_n/(GACA)_m$ sequences are predominantly found in heterochromatic regions (3). Their involvement in sex determination of several mammalian and reptilian species has been discussed (4, 5). The copy number and organization of $(GATA)_n/(GACA)_m$ repeats varies considerably between the genomes of different species. Hybridization of restriction enzyme-digested genomic DNA to cloned $(GATA)_n/(GACA)_m$ sequences or synthetic $(GATA)_4$ and $(GACA)_4$ oligonucleotides, respectively, usually revealed extensive polymorphisms even between individuals, which made these probes a valuable tool for fingerprinting of e.g. human DNA (6).
In the present report, we investigated the occurrence of $(GATA)_n/(GACA)_m$ repeats in the genome of several higher plants. We show that $(GATA)_n$ - as well as $(GACA)_m$ repeats are present in the genome of all angiosperm species examined. However, the complexity of hybridization patterns may vary considerably.

Materials and methods

Six plant species were chosen for the present investigation: Musa acuminata (banana); three unrelated individuals grown in the Palmengarten, Frankfurt, Germany.
Hordeum spontaneum (wild barley); three individuals derived from different accessions (41-3, 41-5, 80-3) collected by the ICARDA Institute, Aleppo, Syria.
Solanum tuberosum (potato); two tetraploid cultivars (Berolina and Bintje; purchased from a local market); one anther-derived dihaploid plant (AH 78.5363; derived from the Institute for Resistance Genetics, Grünbach, Germany).
Brassica napus var. oleifera (oilseed rape); three individuals derived from different cultivars (cv. Optima; cv. Lirama, cv. Topas) provided by the Research Centre for Plant Protection, Lyngby, Denmark.
Cicer arietinum (chickpea); three individuals derived from different accessions (ILC 72, ILC 195, ILC 263) collected by the ICARDA Institute, Aleppo, Syria.
Lens culinaris (lens); three individuals derived from different accessions (ILL 5873, ILL 5876, ILL 5988) collected by the ICARDA Institute, Aleppo, Syria.

DNA was isolated from leaves of individual plants according to a modified version of the CTAB method from Saghai-Maroof et al. (7). 3g of leaf tissue were frozen in liquid nitrogen, ground to a fine powder and subsequently transferred to 15ml of hot (60°C) 2xCTAB extraction buffer (7). After gently swirling the resulting cell lysate for 30min, nucleic acids were isolated by extraction with an equal volume of chloroform/isoamylalcohol (24:1) followed by precipitation with 0.6vol of isopropanol. After centrifugation, pellets were solubilized in TE (10mM Tris-HCl, 1mM EDTA, pH 8), and DNA was further purified by ultracentrifugation in CsCl/ethidium bromide followed by

extraction with TE-saturated 1-butanol and ethanol precipitation. 5µg of DNA from each sample were digested with Alu I, Hinf I or Taq I and the fragments separated on 1.2% agarose gels in TAE buffer (40mM Tris-acetate, 20mM sodium acetate, 1mM EDTA; pH 7.8). The gels were dried on a vacuum gel dryer, denatured, neutralized and hybridized to ^{32}P-labeled $(GACA)_4$ or $(GATA)_4$ probes essentially as described (6). Some gels were used for both probes consecutively. Before reprobing, probes were stripped off the gel by washing in 5mM EDTA at 60°C (2x15min).

Results and discussion

DNA fingerprinting using oligonucleotide probes is a versatile and informative technique for the detection of polymorphisms within animal genomes. In the present examination, we extended our studies to the plant kingdom. Representative results of six species are shown in Fig. 1 and 2. They can be summarized as follows:

1. $(GATA)_n$- as well as $(GACA)_m$-sequences are present in relatively high copy numbers in all species examined (autoradiograms were usually exposed overnight).
2. Band sharing between species is not obvious (see e.g. barley versus potato Fig. 1b, 1c). Thus no inter-specific pattern similarities are detectable. This is even true for different species belonging to the same family (lentil and chickpea, Fig. 1e, 1f; 2e, 2f).
3. The degree of intraspecific pattern similarity strongly depends on the probe. This is especially true for barley (Fig. 1b, 2b) and chickpea (Fig. 1e, 2e). It also varies considerably from species to species. Whereas polymorphism is low or limited in most species/probe combinations, highly heterogeneous patterns result from the hybridization of e.g. oilseed rape and chickpea DNA to the $(GATA)_4$ probe (Fig. 1d and 1e). However, any interpretation of intraspecific variability will require more data and a detailed consideration of the genetics and biology of each species under investigation.
4. Individual-specific patterns within the same variety were not yet observed (not shown).
5. The use of different restriction enzymes (4-base recognition sequences) for the same probe/species combination had no influence on the degree of polymorphism (not shown), although slightly different patterns were detected.

The present results confirm and extend recent data obtained by hybridizing human and M13 minisatellite probes to several plant and yeast DNAs (8-11) as well as preliminary data from our own laboratories (12, 13). Simple repetitive motives such as $(GATA)_n$ and $(GACA)_m$ seem to be present ubiquitously in all eukaryotic organisms. However, their usefulness for DNA fingerprinting depends on the level of intraspecific polymorphism, which can vary considerably between probes used for hybridization and the species investigated. The optimal combination of probe and species has to be determined empirically. Most recent results (13-15) indicate that the collection of informative oligonucleotide probes is probably almost unlimited at least in case of mammalian systems.

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Legend to Fig. 1 and 2: Hybridization of ^{32}P-labeled $(GATA)_4$ (Fig. 1) or $(GACA)_4$ (Fig. 2) to restriction enzyme-digested genomic plant DNAs. DNAs (5µg per lane) were derived from leaves of individual plants from different cultivars or accessions (except banana; three each). For the origins of plants see the Materials and methods section. From left to right:
(a) Musa acuminata; three non-related plants grown in the Palmengarten, Frankfurt, Germany.
(b) Hordeum spontaneum; accessions 41-3, 41-5, 80-3.
(c) Solanum tuberosum; Berolina, Bintje, AH 78.5363.
(d) Brassica napus var. oleifera; Optima, Lirama, Topas.
(e) Cicer arietinum; accessions ILC 72, ILC 195, ILC 263
(f) Lens culinaris; accessions ILL 5873, ILL 5876, ILL 5988.
DNA was digested with Alu I (Fig. 1c, 1d, 1f; Fig. 2b, 2c, 2e, 2f), Hinf I (Fig. 1a, 2a) or Taq I (Fig. 1b, 1e, 2d) and separated on 1.2% agarose gels. The positions of molecular weight markers (in kilobases) are indicated.

Fig 1

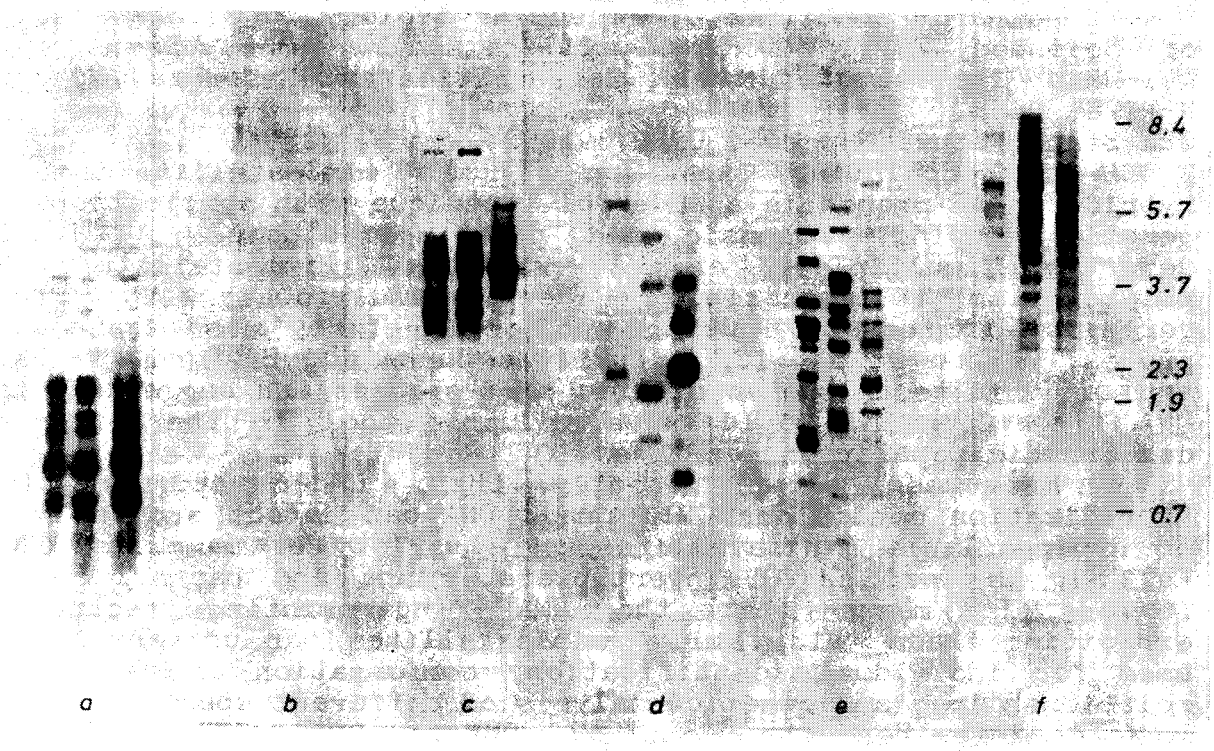

Fig 2