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DNA fingerprinting in sugar beet (*Beta vulgaris*) – identification of double-haploid breeding lines

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Summary. The distribution and abundance of simple repetitive sequences complementary to the synthetic oligonucleotides $(GACA)_4$, $(GATA)_4$, $(GTG)_5$ and $(CA)_8$ in the genomes of several cultivars of *Beta vulgaris* and in the wild beet *B. vulgaris* ssp. *maritima* were investigated. Hybridization experiments revealed that all four motifs were present, though at different abundances, in the genomes of all of the investigated beet cultivars. Considerable intraspecific variation of the resulting DNA fingerprints was observed. The extent of polymorphism depends on the oligonucleotide probe. The most informative banding patterns were obtained with the $(GATA)_4$ probe hybridized to *Hin*fI-, *Hae*III-, or *Rsa*I-restricted DNA, respectively. DNA fingerprinting with $(GATA)_4$ allowed a clear differentiation of double-haploid breeding lines (DH lines). We demonstrated that the application of oligonucleotide probes for DNA fingerprinting is a sensitive tool for genome diagnosis in cultivated beet.

Key words: *Beta* – DH lines – Genome analysis – DNA fingerprinting – Synthetic oligonucleotide probe

Introduction

The application of highly repetitive satellite DNA sequences as species-specific probes for genome diagnosis (Schweizer et al. 1988; Schmidt et al. 1990) as well as the use of single-copy DNA sequences for RFLP mapping of important crop genomes (for review see Tanksley et al. 1989) have greatly facilitated marker-assisted selection approaches in plant breeding. However, the potential of these techniques to differentiate between closely related genotypes of any one specific plant species is still limited. A considerably higher degree of resolution is usually obtained by DNA fingerprinting with polymorphic multi-locus probes. Synthetic oligonucleotide probes complementary to simple repetitive sequences are especially versatile in this respect. Depending on the combination of probe and species, these probes make it possible to distinguish between genotypes at different intraspecific levels in many animal, plant and fungal species (reviewed by Weising et al. 1991b).

The cultivated beet (*Beta vulgaris*) is an important crop with a considerable extent of genetic variability, which has allowed the selection of morphologically different cultivars like Swiss chard (*B. vulgaris* cv *flavescens*), beet root (*B. vulgaris* cv *conditiva*), fodder beet (*B. vulgaris* cv *crassa*) and sugar beet (*B. vulgaris* cv *altissima*) (for review see Barocka 1985). Distinct breeding lines have been established and double-haploid lines (DH lines) have been generated via tissue culture (Bossoutrot and Hosemans 1985). The unequivocal identification and differentiation of these lines is difficult even with molecular markers. We have previously shown that species-specific satellite DNA provides a useful tool for the differentiation of species of the genus *Beta* and for the selection of chromosome addition lines (Schmidt et al. 1990, 1991).

In this paper we present an analysis of the presence and variability of simple repetitive sequences in *B. vulgaris* and introduce the protocol of DNA fingerprint analysis with synthetic oligonucleotide probes for genome diagnosis in cultivated beet. We also describe the design of suitable probe-restriction enzyme combinations for informative fingerprints that

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would allow double-haploid breeding lines of sugar beet to be distinguished.

Material and methods

Plant material

The different varieties and cultivars of *B. vulgaris* were obtained from the Botanical Garden, University of Frankfurt/Main. Leaf material of double-haploid sugar beet lines was kindly provided by Dr. H. J. Kärgel (Institute for Beta Research, Klein Wanzleben, FRG).

Oligonucleotide fingerprinting

Total DNA from leaves was isolated as described previously (Schmidt et al. 1990) and purified by CsCl density centrifugation or chromatography on Qiagen columns (Weising et al. 1991a). DNA was digested with an excess of the appropriate restriction enzyme (6–8 units/μg) under the conditions recommended by the suppliers, separated on 1% agarose gels (5 μg per lane) and transferred onto nylon membranes under standard conditions (Sambrook et al. 1989). Alternatively, gels were dried on a vacuum gel dryer. End-labelling of synthetic oligonucleotide probes with [γ-^{32}P] ATP, prehybridization and hybridization were performed as described by Ali et al. (1986). Melting temperatures of the oligonucleotides were calculated according to Thein and Wallace (1986). Hybridization and stringent washing steps were carried out at $T_m - 5\,°C$. Before reprobing, probes were removed from the dried gels or filters by washing in 5 mM EDTA at 60 °C or in 0.1% SDS at 80 °C, respectively.

Results

Analysis of the genomic distribution of different simple repetitive sequences in beet

The existence and distribution of sequences complementary to $(GACA)_4$, $(GATA)_4$, $(CA)_8$ and $(GTG)_5$ were investigated in several cultivars of *B. vulgaris* (sugar beet, fodder beet and Swiss chard) and its wild subspecies *B. vulgaris* ssp. *maritima*. For the detection of intracultivar or intraspecific variation two individual plants of each cultivar were analyzed.

After hybridization of $(GACA)_4$ to *Hin*fI-digested genomic DNA only a few signals were detected (Fig. 1). While the hybridization patterns consist of a low number of high-molecular-weight bands in all cultivated beets, only very faint bands are visible in the wild beet *B. vulgaris* ssp. *maritima*. A predominant band of about 7.1 kb was observed in all sugar beet samples. Individual plants were discriminated in fodder beet and Swiss chard, but not in sugar beet. Hybridization of $(GATA)_4$ to the same gel resulted in a complex pattern with signals covering a wide range of molecular weights in all *Beta* species. The abundance of $(GATA)_n$-harboring fragments is considerable (Fig. 1) and in a similar range for all of the cultivars analyzed as well as for *B. vulgaris* ssp. *maritima*. The different breeding lines of cultivated beet are clearly distinguishable. Furthermore, there is a distinct intracultivar variation as demonstrated by the different patterns of two individual plants derived from the same breeding line. It is worth noting that a DNA fragment of about 7.1 kb occurs in all sugar beet lanes where a similar-sized fragment also occurs with $(GACA)_4$.

Reprobing of the same gel with $(CA)_8$ and $(GTG)_5$ resulted in very complex hybridization patterns in all of the analyzed *Beta* species (Fig. 1). Both probes detected a number of fragments superimposed on a smear. While the fingerprints of two individuals

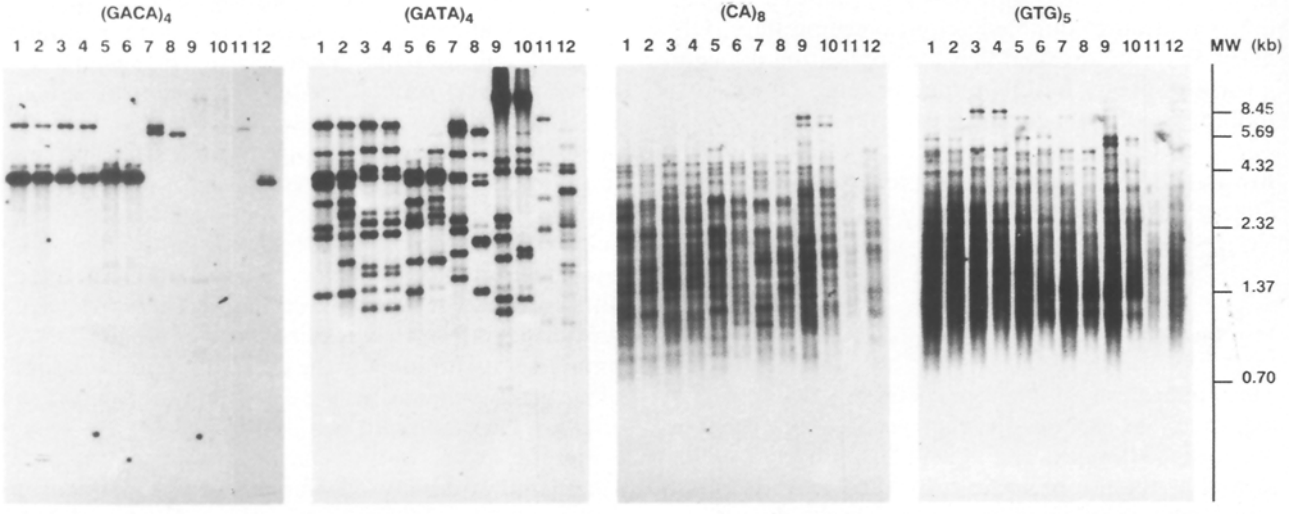

Fig. 1. Presence and polymorphism of various simple sequence motifs in the genomes of different cultivars and species of the genus *Beta*. Total DNA from two individual plants of each cultivar was digested with *Hin*fI. The resulting fragments (5 μg DNA per lane) were separated on 1% agarose gels. After denaturation and neutralization of the DNA the gel was dried and consecutively hybridized with $(GACA)_4$, $(GATA)_4$, $(CA)_8$ and $(GTG)_5$. Molecular weight markers are given in kb. The following *Beta* cultivars and species were investigated: *lanes 1–2, 3–4, 5–6 B. vulgaris* cv *altissima* (two individual plants each of three different breeding lines), *lanes 7–8 B. vulgaris* cv *crassa*, *lanes 9–10 B. vulgaris* ssp. *maritima*, *lanes 11–12 B. vulgaris* cv *flavescens*

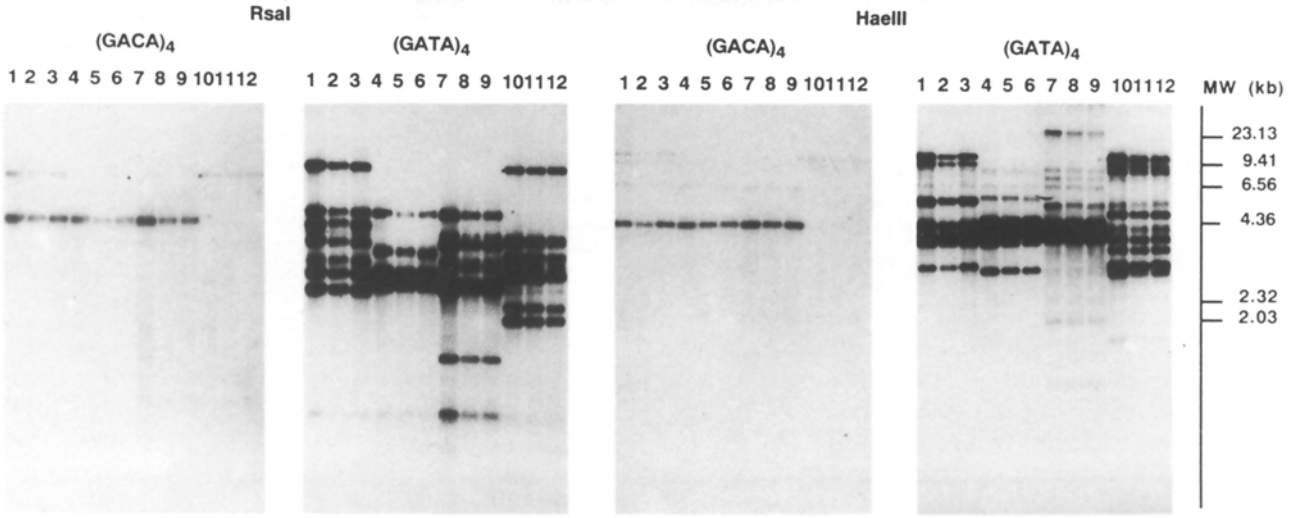

Fig. 2. Identification of double-haploid breeding lines (DH lines) of *B. vulgaris* cv *altissima*. Total DNA from three individual plants from each of four different DH lines was digested with either *Rsa*I or *Hae*III. The resulting fragments (5 µg per lane) were separated on 1% agarose gels and transferred onto nylon membranes. Filters were consecutively hybridized with $(GACA)_4$ and $(GATA)_4$. Molecular weight markers are given in kb

belonging to the same cultivar are identical in many cases, slight but reproducible variations were observed among the different beet cultivars. Both $(GTG)_5$ and $(CA)_8$ might therefore have the potential to give variety-specific fingerprints, though more plants from different origins would have to be tested to prove this prediction. Hybridization with these oligonucleotide probes resulted in even stronger signals than those observed after hybridization with $(GACA)_4$ and $(GATA)_4$. On the basis of the strength of the hybridization signals, the abundance of the repetitive sequence motifs investigated is remarkably different and can be characterized as $(GTG)_5 \geq (CA)_8 > (GATA)_4 > (GACA)_4$. The potential to distinguish between different cultivars of *B. vulgaris* as well as between different plants within a certain cultivar depends on the probe. The use of other restriction enzymes with four-base specificity (*Rsa*I or *Hae*III) had no considerable influence on the complexity and variability of the fingerprint patterns (Fig. 2).

Identification of DH lines of sugar beet (B. vulgaris cv *altissima)*

Since cultivated beet shows strong cross-fertilization (Barocka 1985), high levels of heterozygosity are usually encountered within cultivars. In order to obtain homozygosity in sugar beet, DH lines have been established by tissue culture. We performed a DNA fingerprint analysis of four selected DH lines. To this end, the DNA of three individuals of each line was digested with either *Rsa*I or *Hae*III. The resulting fragments were transferred onto nylon membranes and hybridized to $(GACA)_4$ and $(GATA)_4$, respectively (Fig. 2). As expected from the data shown in Fig. 1, $(GACA)_4$ revealed a few bands only. The fingerprints obtained from individual plants of all four DH lines are identical in each case. On the basis of $(GACA)_4$ fingerprints only DH line no. 4 can be distinguished from the remaining three lines. In contrast, $(GATA)_4$ produces more complex patterns, which allow a clear differentiation between all four DH lines. The fingerprints of different individuals derived from a particular line are identical. This holds true for both restriction enzymes used. Thus, the complexity of the observed fingerprints strongly depends on the probe and less on the restriction enzyme. These results suggest that simple repetitive oligonucleotide probes in general, and $(GATA)_4$ in particular, are suitable tools for the unequivocal identification and characterization of DH lines in sugar beet.

Discussion

Species of the genus *Beta* contain a considerable amount of highly repeated, tandemly arranged satellite DNA sequences in their genomes. Several of these satellite DNA families have been cloned and analyzed in detail with respect to their structure and evolution (Schmidt and Metzlaff 1991; Schmidt et al. 1991). In the present paper we demonstrate by hybridization with oligonucleotide probes $(GACA)_4$, $(GATA)_4$, $(CA)_8$ and $(GTG)_5$ that simple repetitive sequences also contribute significantly to the genome of *Beta* species. As also observed in other plant species

(Weising et al. 1991a, b), the organization and abundance of these short repeats differ from each other, suggesting a considerable degree of copy number variation of repeated motifs at different loci. Notwithstanding their relative abundance, all four probes produced distinct banding patterns, which makes them suitable for DNA fingerprint analyses. Different probes revealed different levels of polymorphisms. The highest degree of variability was observed after hybridization with the oligonucleotide $(GATA)_4$. The high abundance and the high level of polymorphism of its target sequences makes the $(GATA)_4$ probe optimally suited for the characterization and identification of DH lines of cultivated beet.

In some cases, the hybridization of $(GACA)_4$ and $(GATA)_4$ to fragments of similar sizes was observed. These fragments might represent blocks of interspersed $(GACA)_n$ and $(GATA)_m$ motifs as has been described for some animal species (Epplen et al. 1982). The clustering of different simple repetitive motifs is probably a general phenomenon since it has been detected by sequence analysis of simple repeats from various species from different kingdoms (Opstelten et al. 1989; Studer et al. 1991; Condit and Hubbell 1991).

The present results show that DNA fingerprinting is an appropriate approach for genome diagnosis of *B. vulgaris*. Nagamine et al. (1989) used endogenous cDNA probes to generate multi-locus hybridization patterns of *B. vulgaris* cv *altissima*, *B. vulgaris* ssp. *maritima* and *B. nana*. The distinguishing capacity of the majority of these probes, however, was confined to the species level, which is low when compared to that of the $(GATA)_4$ probe used in the present study. Simple repetitive oligonucleotides have several additional advantages over endogenous probes in DNA fingerprint studies. First, they are versatile: their target sequences are present in the genomes of most if not all higher plant species (Weising et al. 1991b). Second, they allow in-gel hybridization, a method that omits blotting and prehybridization steps and is thus faster than conventional techniques (Ali et al. 1986). Third, no cloning steps are necessary to generate the probes. The suitability of a specific probe strongly depends on its base composition and less on the restriction enzyme used for DNA digestion. The optimal probe-enzyme combinations have to be found empirically, a fact that has also been stressed for other plant genera (Weising et al. 1991a).

For the first time specific DNA markers have been developed that reliably allow DH lines which have only been distinguished from each other by isoenzyme analyses so far to be differentiated (H.J. Kärgel personal communication). DNA fingerprinting should also make it possible to estimate the degree of homozygosity in a particular inbreeding line by a comparison of individual plants. As a useful complement to RFLP markers and genome-specific repetitive DNA probes, DNA fingerprinting with simple repetitive oligonucleotides is thus a new and important tool for line identification and the protection of breeders' rights.

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