Theor Appl Genet (1994) 88:775–779

M. Lorenz · A. Weihe · T. Börner

DNA fragments of organellar origin in random amplified polymorphic DNA (RAPD) patterns of sugar beet (*Beta vulgaris L.*)

Received: 15 November 1993 / Accepted: 21 December 1993

Abstract The technique of random amplified polymorphic DNA (RAPD) offers a broad range of applications in the investigation of plant genomes. A promising prospect is the use of RAPD products as genetic markers. We have investigated a possible organellar source of fragments in RAPD patterns of total DNA. Two nearly-isogenic lines of cytoplasmic male-sterile and male-fertile sugar beet (*Beta vulgaris L.*) were subjected to RAPD analysis with six different primers. Total, nuclear, mitochondrial (mt), and chloroplast (cp), DNA from each line were investigated. Reproducible DNA fingerprints could be obtained from both organellar DNAs. Differences in band patterns of mtDNA between cytoplasmic male-sterile and -fertile lines were observed with five out of six primers, whereas different cpDNA patterns were generated by one of the primers. Consequently, the RAPD technique can be used to discriminate between different cytoplasms. Clear evidence is provided for the organellar origin of fragments in genomic (total DNA) RAPD patterns. The consequences of these results for the interpretation of RAPD analyses are discussed.

Key words *Beta vulgaris* · RAPD · Mitochondrial DNA Chloroplast DNA · Cytoplasmic male sterility

Introduction

The development of PCR fingerprint techniques by Williams et al. (1990) (RAPD) and Welsh and McClelland (1990) (AP-PCR) has greatly facilitated the characterization of plant genomes. The method is easy to perform and time saving, and only small amounts of plant tissue are required as compared to RFLP analysis. Applications include analysis of the relationship between varieties, cultivars, and lines (Hu and Quiros 1991; Klein-Lankhorst et al. 1991; Kresovich et al. 1992; Dweikat et al. 1993), estimation of genetic distances between species (Wilkie et al. 1993), and the employment of RAPD fragments as genetic markers (e.g., Devos and Gale 1992).

Parentage analysis has revealed a Mendelian inheritance of RAPD products (e.g., Welsh et al. 1991; Waugh and Powell 1992), and segregation studies have shown the expected dominant nature of RAPD markers (Carlson et al. 1991; Hu and Quiros 1991; Michelmore et al. 1991). DNA bands inherited from one parent to all members of an F_1 progeny have been explained by a homozygous dominant genotype of the parent harbouring these bands (Roy et al. 1992; Heun and Helentjaris 1993). Given a female crossing partner as the origin of "aberrantly"-inherited bands, a possible explanation would postulate that the fragments shared by the female parent and all F_1 progenies originate from organellar DNA which is inherited maternally. However, the RAPD technique has not yet been applied to organellar DNAs and non-Mendelian inheritance of RAPD fragments has not yet been investigated systematically. As the solution of this problem is of great importance for the interpretation of all mapping and segregation data obtained on the basis of RAPD, we have compared RAPD patterns of genomic (total) DNA from two nearly-isogenic lines of sugar beet with patterns derived from purified nuclear, mitochondrial, and chloroplast, sugar beet DNA.

Materials and methods

Plant material

Nearly-isogenic lines 3011 (cytoplasmic male-sterile) and 3012 (male-fertile) of sugar beet (*Beta vulgaris L.*) were kindly provided by Kleinwanzlebener Saatzucht AG (Einbeck, Germany).

Isolation of DNA

Genomic (total cellular) DNA was isolated from sugar beet leaves according to Rogers and Bendich (1985). Mitochondrial (mt) DNA

Communicated by R. Hagemann

M. Lorenz · A. Weihe · T. Börner (✉)
Department of Biology,
Humboldt University, Invalidenstrasse 43,
D-10115 Berlin,
Germany

Table 1 Nucleotide sequence of tested primers

Primer	Nucleotide sequence
1	5′-ACGGTCTTGG
2	5′-GGCATCGGCC
OPB-07	5′-GGTGACGCAG
OPB-10	5′-CTGCTGGGAG
Primer M13	5′-GAGGGTGGCGGTTCT

was isolated as described earlier (Weihe et al. 1991). Chloroplast (cp) DNA was isolated according to Teeri et al. (1985).

For isolation of nuclear DNA, the chromatin pellet obtained during the first step of chloroplast isolation was washed three times with 2% Triton X-100 in lysis buffer, and the DNA extracted with phenol/chloroform. All organellar DNAs were further purified on CsCl-bisbenzimide gradients (Matthews and Widholm 1985).

PCR conditions

Amplification reactions containing 15 ng of template DNA were performed in 50 µl of 10 mM Tris-HCl (pH 8.2), 50 mM KCl, 4.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 20 ng of primer and 2.5 units of *Taq* polymerase (Boehringer), overlaid with 50 µl mineral oil. Amplification was carried out in a BIOMED 60/2 thermocycler (BIOMED, Germany) programmed for 40 cycles of 20 s at 93 °C; 1 min at 36 °C for 10-mer primers [42 °C for $(GATA)_4$ 50 °C for M13-primer]; 20 s extension at 72 °C, and a final cycle of 6 min at 72 °C. Amplification products were analyzed by electrophoresis in 1.2% agarose gels run in 1 × TAE buffer (Maniatis et al. 1982) and detected by ethidium bromide staining.

A "minus-template" control was included in all PCR assays. The nucleotide sequences of the primers used in the experiments are given in Table 1.

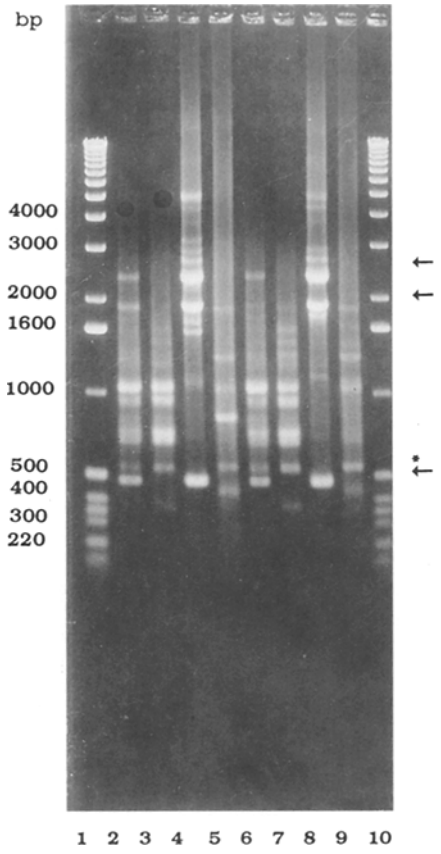

Fig. 1 RAPD analysis of two nearly-isogenic lines of sugar beet (*B. vulgaris L.*) with the core sequence of phage M13 as primer. *Lanes 2–5*, line 3011 (cytoplasmic male sterile); *lane 2*, total DNA; *lane 3*, nuclear DNA; *lane 4*, mtDNA; *lane 5*, cpDNA; *lanes 6–9*, line 3012 (male-fertile); *lane 6*, total DNA; *lane 7*, nuclear DNA; *lane 8*, mtDNA; *lane 9*, cpDNA; *lanes 1 and 10*, 1-kb ladder (Gibco BRL). *Arrows* indicate mtDNA-derived fragments in total DNA; a cpDNA-derived band is marked by an *asterisk*

Results and discussion

To investigate the contribution of organellar DNA to PCR fingerprints obtained with total DNA, we subjected nuclear-, mt- and cp-DNA of two nearly-isogenic sugar beet lines (cytoplasmic male-sterile and male-fertile) to RAPD analysis and compared the resulting fingerprint patterns with those obtained using total DNA. The organellar DNAs were purified on CsCl-bisbenzimide gradients to exclude possible cross-contamination between DNAs of different origin.

Using the core sequence of the protein III gene of phage M13 as primer, reproducible fingerprint patterns were obtained with mtDNA and cpDNA (Fig. 1). Strikingly, the fragments resulting from mtDNA were in the higher-molecular-weight range compared with total and nuclear DNA. This might reflect a lower frequency of the type of inverted repeat necessary for single primer amplifications in mtDNA than in the nuclear genome. There are distinct differences in the mtDNA fingerprint patterns between cytoplasmic male-sterile and male-fertile plants, represented by additional bands of approximately 1 600 and 3 000 bp in the male-sterile plant (Fig. 1, lanes 4 and 8). This is in agreement with experimental evidence indicating that a primary source of cytoplasmic male sterility is extensive rearrangement in the mitochondrial genome (for review see Braun et al. 1992) and with previously-reported differences in restriction fragment patterns between mtDNA of cytoplasmic male-sterile and -fertile sugar beet (Powling and Ellis 1983; Mikami et al. 1984; Weihe et al. 1991). Thus, in addition to mitochondrial RFLP analysis, organellar PCR fingerprinting provides another method for differentiation between cytoplasmic male-sterile and male-fertile cytoplasms. The difference in the fragment patterns of cpDNA between the two lines, mainly the presence of an extra fragment of 800 bp in the male-sterile plant, is surprising (Fig. 1, lanes 5 and 9). Chloroplast genomes within one species are usually well conserved. On the other hand, single point mutations may exist which cannot be revealed easily by DNA restriction analysis. A polymorphism at one *Hind*III site has been detected in cpDNA between male-sterile and male-fertile sugar beet (Mikami et al. 1984).

At least three fragments are shared by total- and mtDNA RAPD patterns which are absent from the nuclear fingerprint pattern of both lines, indicating that these bands in the total DNA preparation are derived from mtDNA (see arrows in Fig. 1). And, in fact, these fragments represent the most prominent bands in the mtDNA patterns.

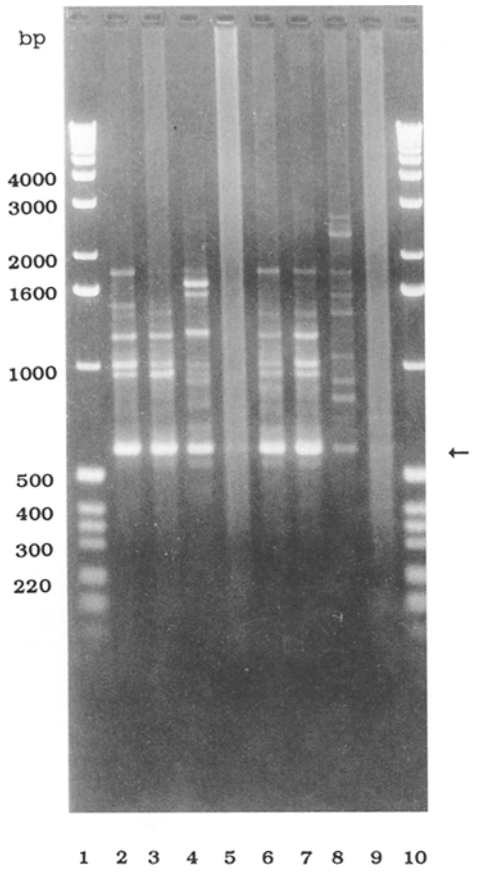

Fig. 2 Segregation of RAPD patterns produced by primer $(GATA)_4$. The lanes are in the order of Fig. 1. A 600-bp fragment observed both in nuclear and mtDNA patterns is indicated by an *arrow*

One of the fragments of the cpDNA fingerprints in both lines appears also in the total- and nuclear-DNA-derived patterns (asterisk in Fig. 1). The most obvious explanation would be a slight contamination of the nuclear DNA by cpDNA, which cannot be excluded even after CsCl-gradient purification. Another source of cpDNA RAPD fragments in nuclear DNA, and thus total DNA, can possibly be found in organellar DNA sequences transferred to the nucleus (cf. Gray 1991).

The PCR fingerprint patterns derived from nuclear DNA are very similar between the cytoplasmic male-sterile and male-fertile line. Minor differences are most likely due to the fact that the lines are not fully isogenic.

We have also employed simple tandem repeats as primers for RAPD analysis. Figure 2 shows the results obtained with $(GATA)_4$. Also with this type of primer, the fingerprint patterns obtained with mtDNA were highly different between the male-sterile and male-fertile line. There is one fragment of about 600 bp (marked by an arrow) which appears in the patterns of mitochondrial, nuclear, and total DNA in similar quantity. In this case, it could be assumed that a sequence of nearly-identical length is amplified from mtDNA and nuclear DNA. No clear band pattern was produced using $(GATA)_4$ as primer and cpDNA as template.

To investigate whether the appearence of cytoplasmic DNA sequences is a common feature in RAPD analyses of total DNA we have tested further primers. Figure 3 shows the results obtained with four arbitrarily-selected 10-mers. As in the case with the M13-primer, high-molecular-weight fragments were obtained with mtDNA as template. However, dependent upon the primer used, the mtDNA RAPD patterns varied in the extent of differences between the male-sterile and male-fertile line. Thus, primers 1, 2, and OPB-07 produced relatively-similar patterns, whereas primer OPB-10 generated larger differences in the range of 1 600 to 2 000 bp (Fig. 3, lanes 13 and 17). The PCR fingerprint obtained with primer OPB-07, showing an mtDNA-derived band of about 600 bp in the total DNA but not in the nuclear DNA pattern, represents another striking example of the cytoplasmic origin of bands in "genomic" RAPD patterns (see arrow in Fig. 3).

Our results indicate that organellar (mt and cp) genomes can be discriminated by PCR fingerprinting. Furthermore, they demonstrate that, at least in sugar beet, and depending on the primer used for PCR amplification, a number of fragments in "genomic" RAPD patterns originate from mtDNA and to less extent from cpDNA. Similar observations have been made by DNA fingerprint studies on organellar DNAs using $(GATA)_4$ and other simple repeats as hybridisation probes (Beyermann 1992). Thus, when certain fragments appear both in the female parent and in all of the descendents of a cross, this may be due to maternal inheritance.

The question arises why not *all* fragments detectable in PCR fingerprints of mtDNA and cpDNA (and also nuclear DNA) are present in fingerprint patterns of total DNA. The contribution of organellar genomes to total cellular DNA varies between species and between cell types. It can be estimated to be about 1% for mtDNA and about 10% for cpDNA (Herrmann and Possingham 1980; Ward et al. 1981; Bendich and Gauriloff 1984). The finding that, if any, only prominent bands of organellar origin are detectable in PCR fingerprints of genomic DNA could be the result of competition for primer binding between suitable sites in the DNAs of different sources. Earlier experiments with mixed samples of two different genomic DNAs gave similar results (data not shown). It is well known that minor quantities of a specific fragment can be detected within a large amount of non-target DNA, if PCR is performed in the "traditional" way to amplify one specific DNA sequence by two different primers. However, if the RAPD technique is applied to detect specific bands in fingerprint patterns of two different total DNAs mixed at various ratios in one sample, a strikingly lower sensitivity is observed (Lorenz et al., in preparation).

Our results indicate that reciprocal crosses or F_2 segregation analyses are necessary for resolving the question of whether "band sharing" between the female parent and F_1 progenies is due either to a homozygous genotype of one of the parents, as discussed by others (Heun and Helent-

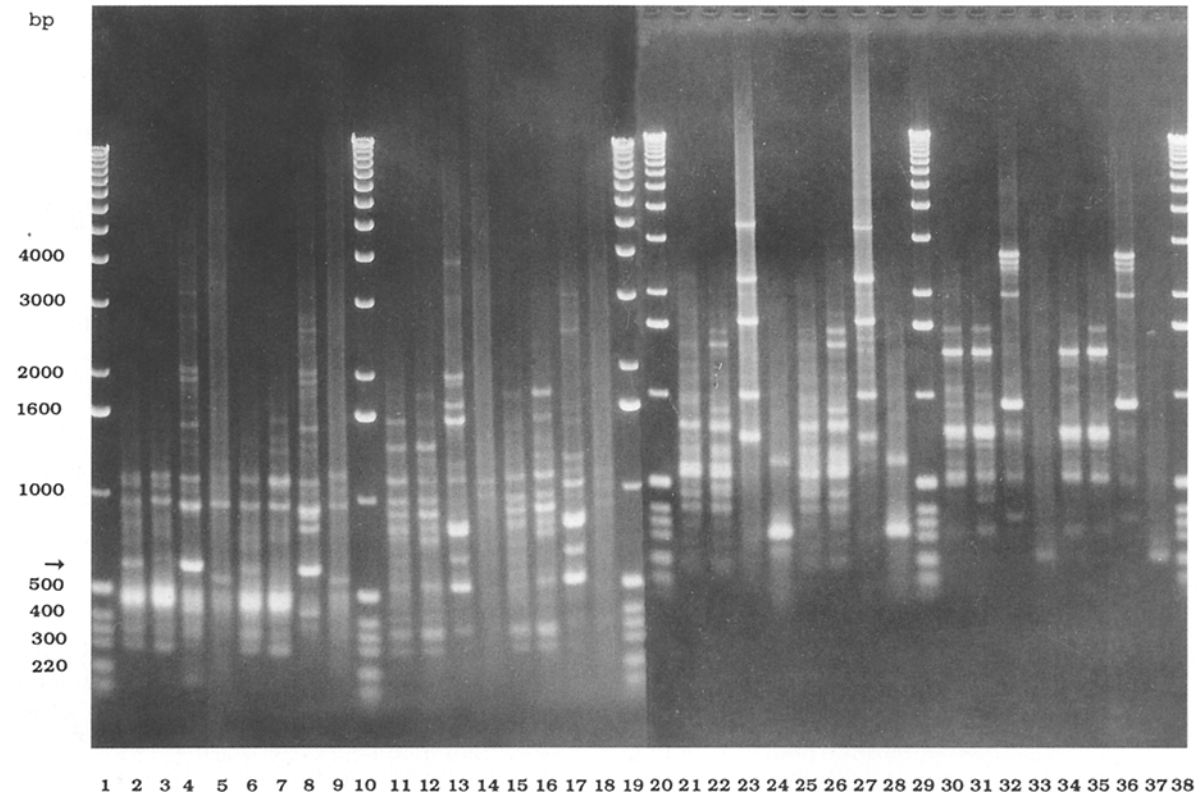

Fig. 3 RAPD patterns of the two sugar beet lines produced by primer OPB-07 (*lanes 2–9*), primer OPB-10 (*lanes 11–18*), primer 1 (*lanes 21–28*) and primer 2 (*lanes 30–37*). Order of lanes identical to those from Fig. 1. *Lanes 1, 10, 19, 20, 29 and 38*, 1-kb ladder (Gibco BRL). An mtDNA-derived fragment in total DNA is indicated by an *arrow*

jaris 1993), or to maternal inheritance (indicated by organellar fragments). The findings presented here might well be a common feature in RAPD analyses applicable to other, if not all, plant species. Whenever aberrant inheritance of RAPD fragments is observed the contribution of cytoplasmic amplification products should be considered a matter of serious concern having important consequences both for the interpretation of results in segregation analysis and for the use of RAPD fragments as genetic markers.

References

Bendich AJ, Gauriloff LP (1984) Morphometric analysis of cucurbit mitochondria: the relationship between chondriome volume and DNA content. Protoplasma 119:1–7

Beyermann B (1992) DNA-Fingerprinting von Pflanzengenomen auf der Basis von simplen tandem repetitiven Sequenzen. Thesis, Humboldt University, Berlin

Braun CJ, Brown GG, Levings III CS (1992) Cytoplasmic male sterility. In: Herrmann RG (ed) Plant gene research: cell organelles. Springer-Verlag, Wien New York, pp 219–245

Carlson JE, Tulsieram LK, Glaubitz JC, Luk VWK, Kauffeldt C, Rutledge R (1991) Segregation of random amplified DNA markers in F_1 progeny of conifers. Theor Appl Genet 83:194–200

Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84:567–572

Dweikat I, Mackenzie S, Levy M, Ohm H (1993) Pedigree assessment using RAPD-DGGE in cereal crop species. Theor Appl Genet 85:497–505

Gray MW (1991) Origin and evolution of plastid genomes and genes. In: Bogorad L, Vasil IK (eds) The molecular biology of plastids. Academic Press, San Diego, pp 303–330

Herrmann RG, Possingham JV (1980) Plastid DNA - the Plastome In: Reinert J (ed) Chloroplasts. Springer-Verlag, Berlin Heidelberg New York , pp 45–96

Heun M, Helentjaris T (1993) Inheritance of RAPDs in F_1 hybrids of corn. Theor Appl Genet 85:961–968

Hu J, Quiros CF (1991) Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep 10:505–511

Klein-Lankhorst RM, Vermunt A, Weide R, Liharska T, Zabel P (1991) Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). Theor Appl Genet 83:108–114

Kresovich S, Williams JGK, McFerson JR, Routman EJ, Schaal BA (1992) Characterization of genetic identities and relationships of *Brassica oleracea* L. via random amplified polymorphic DNA assey. Theor Appl Genet 85:190–196

Maniatis T, Fritsch E, Sambrook J (1982) Molecular cloning: a laboratory manual: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Matthews BF, Widholm JM (1985) Organellar DNA compositions and isozyme expression in an interspecific somatic hybrid of *Daucus*. Mol Gen Genet 198:371–376

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregation analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Mikami T, Sugiura M, Kinoshita T (1984) Molecular heterogenity in mitochondrial and chloroplast DNA from normal and male-sterile cytoplasms in sugar beet. Curr Genet 8:319–322

Powling A, Ellis THN (1983) Studies on the organelle genomes of sugar beet with male-fertile and male-sterile cytoplasms. Theor Appl Genet 65:323–328

Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol Biol 5:69–76

Roy A, Frascaria N, MacKay J, Bousquet J (1992) Segregating random amplified polymorphic DNAs (RAPDs) in *Betula alleghaniensis*. Theor Appl Genet 85:173–180

Teeri TH, Saura A, Lokki J (1985) Insertion polymorphism in pea chloroplast DNA. Theor Appl Genet 69:567–570

Ward BL, Anderson RS, Bendich AJ (1981) The size of the mitochondrial genome is large and variable in a family of plants (Cucurbitaceae). Cell 25:793–803

Waugh R, Powell W (1992) Using RAPD markers for crop improvement. Trends Biotechnol 10:186–191

Weihe A, Dudareva NA, Veprev SG, Maletsky SI, Melzer R, Salganik RI, Börner T (1991) Molecular characterization of mitochondrial DNA of different subtypes of male-sterile cytoplasms of the sugar beet *Beta vulgaris L.* Theor Appl Genet 82:11–16

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Welsh J, Honeycutt RJ, McClelland M, Sobral BWS (1991) Parentage determination in maize hybrids using the arbitrarily-primed polymerase chain reaction (AP-PCR). Theor Appl Genet 82: 473–476

Wilkie SE, Isaac PG, Slater RJ (1993) Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*. Theor Appl Genet 86:497–504

Williams JGK, Kubilek AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535