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Intraspecific diversity of sugar beet (*Beta vulgaris*) mitochondrial DNA

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Summary. Mitochondrial (mt) DNA, isolated from different sugar beet populations, was analyzed using BamHI and EcoRI restriction enzymes. It was shown that plants possessing the new mtDNA types are revealed among O-type fertilizers quite frequently. Among cytoplasmic male sterile (cms) plants, which evolved during cultivation of O-type fertilizers, plants with altered mt genome were found.

Key words: *Beta vulgaris* – Mitochondrial DNA – Cytoplasmic male sterility

Introduction

It is now generally accepted that genus *Beta* includes 15 species classified into three sections: *Beta*, *Corrolinae*, and *Pattelaris* (Zosimovich 1968). Restriction analysis of the chloroplast (cp) DNA from both cultivated and wild species revealed variations not only between species belonging to different sections (Samoylov et al. 1986) but also between species within the same section (Kishima et al. 1987).

Within fertile sugar beet populations, sterile forms may be found (Owen 1945). Restriction analysis of the mt DNA held in a few laboratories revealed differences in mt DNA patterns between fertile and sterile plants (Powling 1982; Powling and Ellis 1983; Mikami et al. 1984c; Samoylov et al. 1986). In contrast, in all but one case (Mikami et al. 1984a, b) the corresponding cp DNAs were identical (Powling 1982; Powling and Ellis 1983; Samoylov et al. 1986).

Comparative analysis of mt DNAs from wild cms lines of sugar beet revealed variations in restriction fragment patterns between plants. In contrast, mt DNAs from fertile plant were found to be identical (Mikami et al. 1985). Moreover, mt DNA diversity was discovered among fertile plants – sterility maintainers (O-type fertilizers) (Samoylov et al. 1987).

To obtain detailed information about mt DNA variability in sugar beet, we performed restriction analysis of mt DNA preparations isolated from the individual fertile and sterile plants.

Materials and methods

Plant material involved in an analysis

Cultivars. Belotserkovskaya single seed (BL 34 and BL 45); Kirgizskaya single seed (KR 25); Ivanovskaya single seed (IV); Jaltyshkovskaya single seed (JL);

Sterility maintainer lines (O-type fertilizers). KR; BL 34; BL 45; IV 117; IV 194; IV 220; IV 225;

Cms-lines. IVs 117; IVs 194; IVs 269;

Spontaneous cms-lines selected from O-type fertilizer populations. IV 220s; IV 225s1; IV 225s2; semi-sterile form IV 346;

Fertile forms. IV 697; Mezhotninskaya multi seed MZ 80; BL 34/26; BL 34/20

Mt DNA analysis

Mitochondria were isolated from beet roots according to the published procedures (Wilson and Chourey 1984) with insignificant modifications. Restriction endonucleases were obtained from NPO "Ferment" (Vilnius, USSR). DNA digestions were performed according to the supplier's instructions. The fragments after digestion were separated in 0.8% agarose gels. The fragments of the lambda phage DNA digested with HindIII, EcoRI, or PstI were used as molecular weight markers.

Transfer of the separated fragments to nitrocellulose filters and hybridization was done as described by Southern (1975). The probes were labelled using nick-translation. The cloned genes coding the following proteins or RNAs were used as

probes: cytochrome oxidase subunit II (cox II), 18S + 5S rRNA (rrn18 + rrn5), 26S rRNA (rrn26) from mt DNA of *Zea mays* (isolated by C. S. Levings, III, obtained from Dr. V. I. Negruk); photosystem I apoprotein (psaA) and large subunit of ribulose-1, 5-bisphosphate carboxylase/-oxygenase (rbcL) from cp DNA of spinach (gift of Prof. R. G. Herrmann). The two chloroplast genes were used because the mt DNA contains sequences homologous to that of the cp DNA (Brennicke et al. 1983; Hause et al. 1986).

Results

Restriction analysis of mt DNAs from fertile sugar beet plants

Restriction patterns of mt DNAs from O-type fertilizers (lines IV 194, IV 117, BL 34) and from their progenitor plants (cultivars BL 34, IV) are shown in Fig. 1 a. It is evident that every O-type fertilizer has its own specific mt DNA restriction pattern differing from both corresponding progenitor and other O-type fertilizers. A similar comparison made for the O-type fertilizer line BL 45 and its progenitor cultivar also revealed differences. The fertilizer mt DNA contains an additional fragment (4.46 kbp, Fig. 1 b). The fertile lines BL 45 and KR had different mt DNA patterns. The mt DNA from the first cultivar lacked three fragments (11.1, 7.25, and 4.26 kbp) (Fig. 2 a). Restriction analysis of mt DNAs from the 14 individual plants originated from the BL 34 cultivar (Fig. 3) allowed classification into two groups, one containing a mt DNA identical to that of the original cultivar except for increased quantity of the single 4.16-kbp fragment, others having more differences.

Restriction patterns of the mt DNAs from the cms sugar beet plants

We analyzed the mt DNA from the cms lines selected from O-type fertilizer populations and found that they differ from that of the original plants. Figures 2 b and 5 a demonstrate two mt DNA types characteristic for fertile and sterile plants as revealed after EcoRI and BamHI digestion. The mt DNA of the sterile plants was identical to that described earlier for Owen-type cytoplasm (Fig. 1 a). Among the cms plants selected from the IV cultivar, differences in mt DNA patterns were also found, e.g., the mt DNA from the one plant contained an additional 4.57-kbp fragment as compared to the standard Owen cytoplasm mt DNA (Fig. 5 a).

Blot hybridization analysis of the mt DNA

Blots of the gels containing BamHI-digested mt DNA of the fertile and sterile plants BL 34/20, BL 34/26, and IVs 117 were hybridized with the ^{32}P-labelled probes (see "Materials and methods" for the sequences used as probes). A cox II probe distinguished three mt genotypes; one hybridization band was common to all

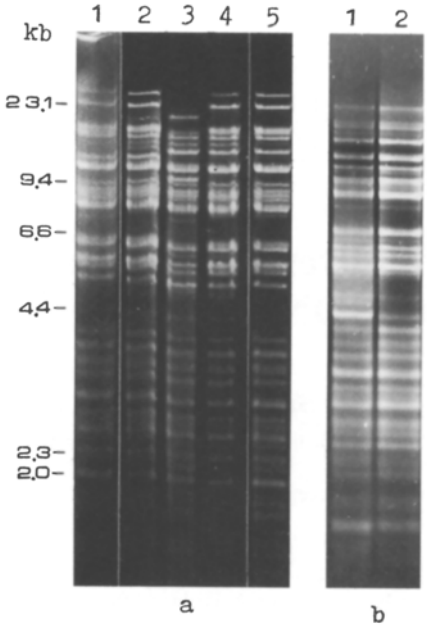

Fig. 1 a and b. Electrophoregrams of BamHI fragments of sugar beet mt DNA **a** *1* – IV; *2* – BL 34 (O-type); *3* – BL 34; *4* – IV 117 (O-type); *5* – IV 194 (O-type); **b** *1* – BL 45 (O-type); *2* – BL 45. The numbers on the left side of the electrophoregram show the sizes of Hind III-fragments of lambda DNA in kbp

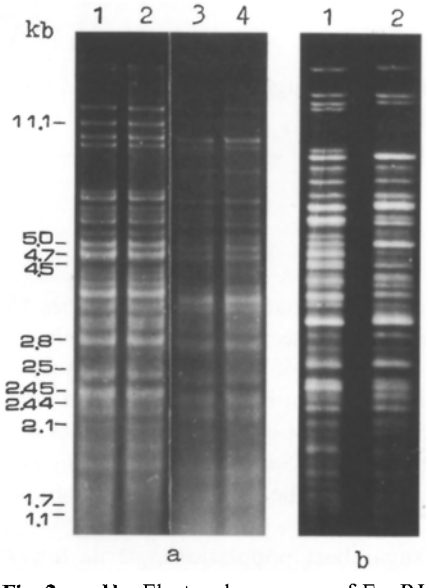

Fig. 2 a and b. Electrophoregrams of EcoRI fragments of sugar beet mt DNA **a** *1, 2* – KR 25; *3,4* – BL 45; **b** *1* – IV 220 (O-type); *2* – IV 220s. The numbers on the left side of the electrophoregram show the sizes of PstI fragments of lambda DNA in kbp

mt DNAs analyzed, the 1.7-kbp fragment was present in the mt DNAs isolated from BL 34/26, IV 177, BL 34/20, IV. The 2.5- and 1.5-kbp fragments were present in the mt DNAs from BL 34/20, IV, and the 3.8-kbp fragment was characteristic for the IVs 117 only. The rbcL, psaA, and rrn18 + rrn5 probes gave hybridization bands with molecular weights of 3.6, 2.3, and 5.2 kbp, respectively,

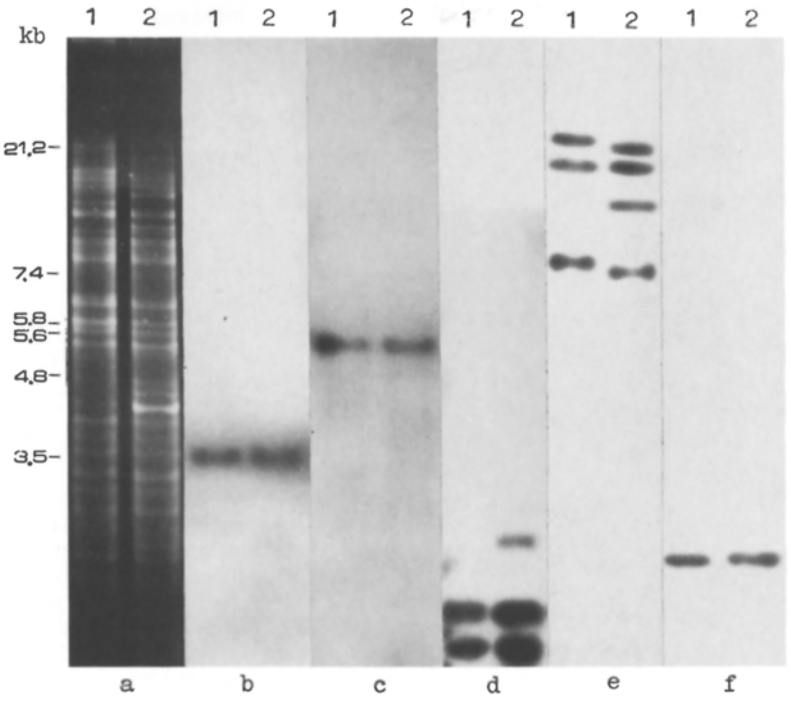

Fig. 3 a–f. Southern blot (**b, c, d, e, f**) of mt DNA digested with BamHI (**a**) from fertile forms: *1* – BL 34/20; *2* – BL 34/26; probed with radiolabed rbcL, rrn18+rrn5, coxII, rrn26, psaA genes. The numbers on the left side of the electrophoregram show the sizes of HindIII fragments of lambda DNA in kbp

for the mt DNAs from both fertile and sterile lines. The rrn26 probe revealed four genotypes after hybridization with BamHI-digested mt DNAs. It gave three fragments (22.3, 16.9, and 8.3 kbp) with the mt DNA from the BL 34/20, four fragments (19.4, 16.9, 11.4, and 7.6 kbp) with the mt DNA from the BL 34/26, four fragments (22.3, 16.9, 8.3, and 6.0 kbp) with the mt DNA from the IV, and three fragments (23.0, 11.4, 6.0 kbp) with the mt DNA from the IVs 117. Therefore, some fragments were common for different lines (e.g., the 22.3-kbp fragment was found to be common for the BL 34/20 and IV 117; the 16.9-kbp fragment was present in BL 34/20, BL 34/26, and IV; the 11.4-kbp fragment was found in BL 34/26 and IV).

Discussion

In the early works devoted to mt DNA analysis, only one type has been described for sugar beet (Powling 1982; Powling and Ellis 1983; Mikami et al. 1985). Later, it was shown that there exist at least two types of mt DNA among the fertile sugar beet plants (Samoylov et al. 1986), the first identical to that described earlier, and the second found in the Janash cultivar of Polish origin, and identical to that of the plants with the Owen cytoplasm. The similar diversity of the mt DNA restriction patterns was found in *Vicia faba* (Negruk and Kaushik 1988) and *Triticum aestivum* (Davidenko et al. 1988).

Unexpectedly, wide diversity of the mt DNA was found in the populations of the O-type fertilizers (Samoylov et al. 1987). This finding allowed us to screen these populations for new chondrion types. Only those fertile plants that are double homozygotes (xxzz) and have normal cytoplasm are capable of maintaining sterility (Negovsky 1968). Such O-type fertilizers are phenotypically identical to the usual fertile plants possessing either X or Z dominant factors. Our results show clearly that the possibility of selecting fertile plants with different cytoplasmons could be realized only using material that is homozygous for X and Z factors.

Our investigation has shown that the O-type fertilizers have specific BamHI restriction patterns of mt DNAs, as compared to that of the original cultivars (Fig. 1 a, b). Therefore, we concluded that they are new mitochondrial mutants.

It is possible to trace mt genome evolution in the course of genetic manipulations and breeding only if the cultivar or variety genealogy is well documented. Among the forms we analyzed, the following groups are of especial interest: (a) JL, KR, and IV; (b) BL 34, BL 45, and their derivatives BL 34/20 and BL 34/26. The IV and KR cultivars were obtained using individual selection from the JL cultivar. All these cultivars have mt DNA identical to that of *B. maritima* as revealed by the BamHI and PstI restriction analysis (Samoylov et al. 1986). BL 34 and BL 45 have common origin, and their mt DNA restriction patterns are also identical. Nevertheless, the two fertile forms BL 34/20 and BL 34/26, which were selected from the BL 34 cultivar, differ in this respect both from each other and from the original cultivar. The latter was shown using restriction patterns of the total mt DNA along with blot hybridization with the rrn26 and cox II probes.

We do not know the genealogy of the sugar beet cultivar Janash, but from the similarity found between its mt DNA patterns and that of cms-lines, we assume that

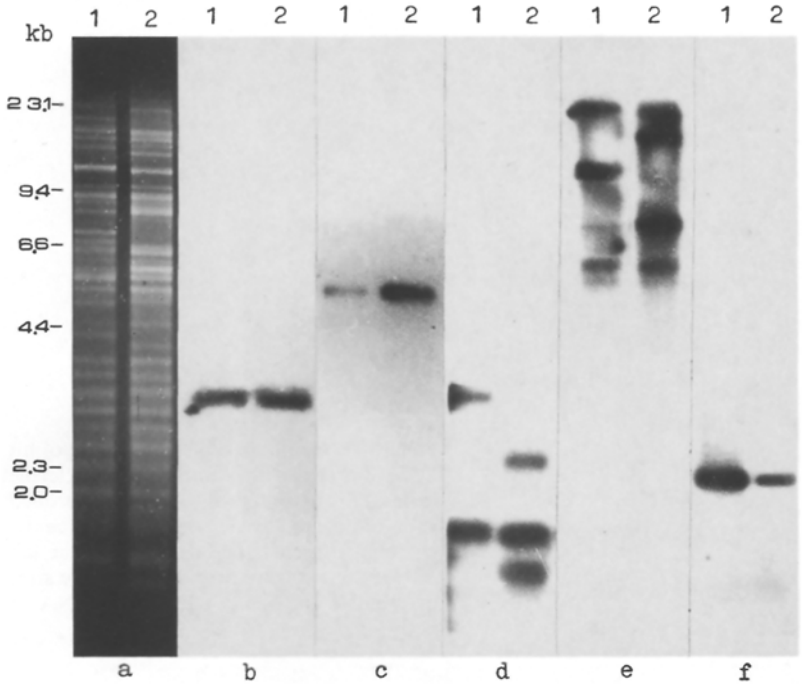

Fig. 4 a–f. Southern blot (**b, c, d, e, f**) of mt DNA digested with BamHI (**a**) from: *1* – IVs117; *2* – IV; probed with radiolabeled rbcL, rrn18+rrn5, coxII, rrn26, PsaA genes. The numbers on the left side of the electrophoregram show the sizes of EcoRI fragments of lambda DNA in kbp

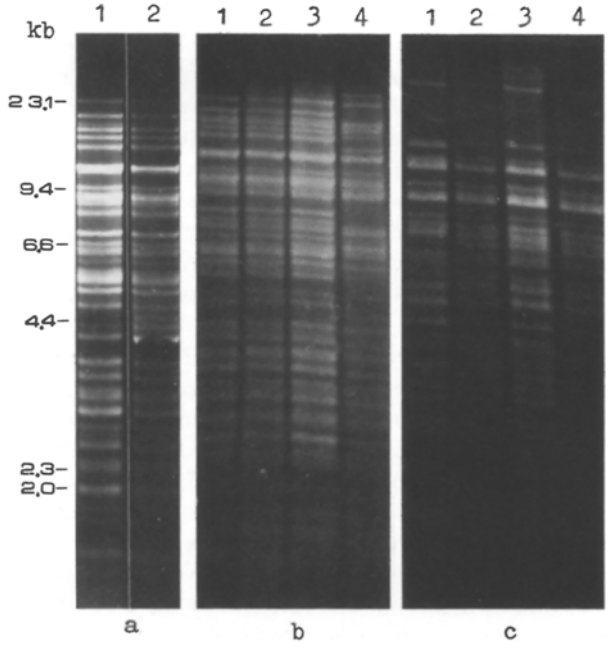

Fig. 5 a–c. Electrophoregrams of BamHI (**a, b**) and HindIII (**c**) fragments of sugar beet mt DNA. **a** *1* – IVs1 255; *2* – IVs2 255; **b** *1* – MZ80; *2* – IVs 269; *3* – IV 346; *4* – IV 697; **c** *1* – IV 697; *2* – IV 346; *3* – IVs 269; *4* – MZ80. The numbers on the left side of the electrophoregram show the sizes of HindIII fragments of lambda DNA in kbp

they had the same cytoplasm source. Due to the fact that in sugar beet, fertility restoration is not necessarily associated with the mt DNA changes (Fig. 5 b, c), it is possible that the Janash cultivar was selected from the material in which the reversion of the cms trait took place either as a result of a spontaneous mutation in mt DNA or due to introduction of the fertility restorer genes. The fact that the restoration of fertility may not be associated with mt DNA changes was also shown for *Paseolus* (MacKenzie et al. 1988), *Zea mays* (Escote-Carlson et al. 1988), and *Brassica* (Makaroff and Palmer 1988).

It is also possible to screen for altered mt genomes among the existing cms populations. Their spontaneous occurrence is very rare, which is why, since the discovery of the cms trait in sugar beet, only the single type of sterile cytoplasm is available (Barocka 1985). At the same time, it was reported that up to 10% of the O-type fertilizer progeny express the cms trait (Hornsey 1973). We have shown that such cms lines can be used for screening genetically new cytoplasmons. Mt DNA restriction analysis has shown that most of these plants contain the mitochondrion, which differs from the parental type and is identical to that characteristic for the Owen-type cms (Fig. 5 a). This fact is well known not only for sugar beet (Powling 1982; Powling and Ellis 1983; Mikami et al. 1984c, 1985), but also for *Zea mays* (Thompson et al. 1980; Spruill et al. 1981; Levings and Pring 1977), *Sorghum* (Conde et al. 1982), *Triticum* (Berenice et al. 1986), *Oryza* (Yamaguchi and Kakiuchi 1983), *Helianthus* (Leroy et al. 1985), *Vicia* (Boutry and Brignet 1982), and *B. maritima* (Boutin et al. 1987).

In these experiments, in addition to general restriction pattern changes, we also observed rearrangements of the rrn26 and cox II genes or their parts (Fig. 4). Among the analyzed plants, one containing an additional 4.57-kbp fragment was found (Fig. 5 a). A similar result was reported earlier (Samoylov et al. 1987). In this work the

plant was found in which two BamHI fragments (5.7 and 4.2 kbp) were absent. The new mt DNA type was also found in the population of *B. maritima* (Mann et al. 1989) and *B. vulgaris* (Hallden et al. 1988).

To sum up, in this work we were able: (a) to perform an efficient screening of the fertile sugar beet plants with new mt DNA types; (b) to use O-type fertilizers for the screening of the new cms variants with an altered mt DNA; (c) to show intercultivar and intrapopulational variability of the mt DNA in sugar beet.

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