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The use of mitochondrial DNA polymorphism in the classification of individual onion plants by cytoplasmic genotypes

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Summary. Individual plants of a Japanese onion variety 'Sapporo-ki', which is characterized by the occasional occurrence of male-sterile plants, have been investigated for mitochondrial (mt) DNA polymorphism. Male-fertile and the Jones' cytoplasmic male-sterile (CMS) onions were also included for comparison. Southern blot hybridization with *rrn26, cox-I, cox-II, cob, atpA* and *atp9* genes as probes revealed the two classes of mtDNA variation within a population of 'Sapporo-ki': Out of the 41 plants examined 19 contained mtDNA typical of male-fertile plants, and 22 individuals contained mtDNA typical of the Jones' CMS genotype. Our results thus indicate that the use of the mitochondrial gene probes may greatly facilitate the classification of individual plants by cytoplasmic genotypes.

Key words: Onion – *Allium cepa* – Cytoplasmic male sterility – Mitochondrial DNA

Introduction

Cytoplasmic male sterility (CMS) is widespread among the plant kingdom and provides a convenient and proprietary means to produce hybrid seed (Newton 1988). In onion (*Allium cepa* L.), CMS was first observed by Jones (Jones and Emsweller 1936), who found a male-sterile plant in a population of the variety 'Italien Red'. This material has given rise to nearly all of the CMS lines presently used by breeders in both Japan and the United States.

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The mitochondrial (mt) DNAs of the male-fertile (normal) and the Jones' CMS onions have been reported to give distinctive restriction profiles (de Courcel et al. 1989; Holford et al. 1991). This allows a cytoplasm to be identified quickly. If it would be able to develop the pairs of male-sterile/maintainer lines from the locally adapted cultivar, they could directly be used as the seed parent in breeding programmes (Dowker 1990). A Japanese open-pollinated variety 'Sapporo-ki' has attracted our interest because of the occasional occurrence of male-sterile plants. The purpose of the study presented here was to survey the cytoplasmic genome variation in onion var 'Sapporo-ki' by restriction fragment length polymorphism (RFLP) analysis of mtDNA. The expectation was that the data will aid in the classification of individual plants by cytoplasmic genotypes.

Materials and methods

Plant material

Analyses were carried out on individual plants of two Japanese open-pollinated varieties 'Sapporo-ki' and 'Imai-wase' from the onion germ plasm collection at Hokkaido National Agricultural Experiment Station, Sapporo, Japan. A pair of CMS/maintainer lines, W202A (carrying the Jones' CMS-S cytoplasm) and W202B (N cytoplasm), were also included for comparison. These materials were kindly provided by Dr. W.H. Gabelman, University of Wisconsin, Madison, Wis. USA.

Isolation of mtDNA

Mitochondria and mtDNA were isolated by a combination of differential centrifugation and DNase I treatment according to Holford et al. (1988). Sprouting onion leaves (10 g) were homogenized in 40 ml of homogenization buffer (10 m*M* TES, 0.5 *M* mannitol, 10 m*M* EGTA, 0.2% BSA and 0.05% cysteine,

pH 7.2). The DNA obtained was purified by phenol-chloroform extraction, ethanol precipitation and RNaseA-treatment.

mtDNA analysis

Restriction endonuclease digestions were performed under conditions specified by the suppliers. The DNA was electrophoresed on agarose slab gels buffered with 40 m*M* TRIS-HCl, 20 m*M* Na-acetate, 2 m*M* EDTA and 18 m*M* NaCl, pH 8.0. The gels were Southern blotted onto nylon membranes (Hybond N, Amersham) according to the manufacturer's recommendations. The filter was further hybridized overnight at 42 °C with constant shaking in the hybridization buffer [enhanced chemiluminescence (ECL) method, Amersham] containing the labelled probe. The hybridized blot was then rinsed twice with the first washing solution (6 *M* urea, 0.4% SDS and 0.5× SSC) for 20 min at 42 °C, followed by two rinses of the second washing solution (2× SSC) for 5 min at room temperature. Labelling of probe DNA and visualization of the probe-target DNA hybrid were carried out by the ECL method according to the supplier's instructions.

Mitochondrial genes used in the hybridization studies were: pea *rrn26* (480-bp *Eco*RI-*Sal*I fragment; Huh and Gray 1982), sugarbeet *cox-I* (1,500-bp *Eco*RI fragment; Senda et al. 1991), sugarbeet *cox-II* (400-bp *Sal*I-*Hin*dIII fragment; Senda et al. 1991). Wheat *cob* (700-bp *Hin*dIII-*Eco*RI fragment; Boer et al. 1985), pea *atpA* (800-bp *Eco*RI-*Bam*HI fragment: Morikami and Nakamura 1987a), and pea *atp9* (700-bp *Xho*I-*Eco*RV fragment; Morikami and Nakamura 1987b).

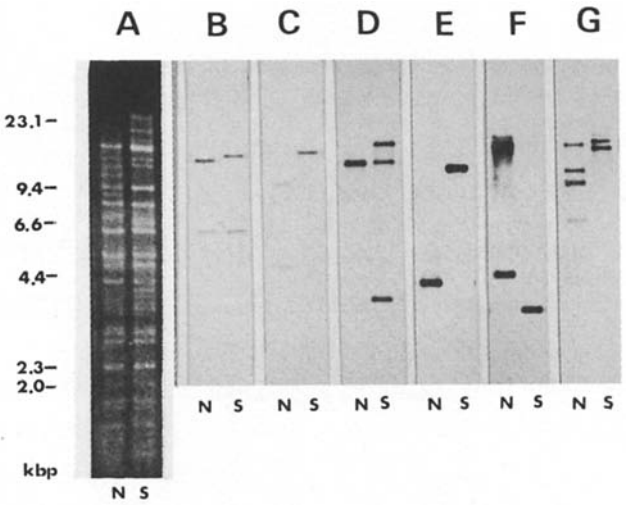


Fig. 1A–G. Southern hybridization analysis of mtDNA from W202B (*N*; N cytoplasm) and W202A (*S*; CMS-S cytoplasm). MtDNA was cut with the *Bam*HI enzyme and electrophoresed in 0.8% agarose gel. *Panel A* shows the restriction pattern after ethidium bromide staining of the gel. The gel was blotted onto a nylon membrane filter and hybridized with mitochondrial gene probes. The probes were: *B rrn26* of pea, *C cox-I* of sugarbeet, *D cox-II* of sugarbeet, *E cob* of wheat, *F atpA* of pea; *G atp9* of pea. Size markers are indicated in kbp

Results and discussion

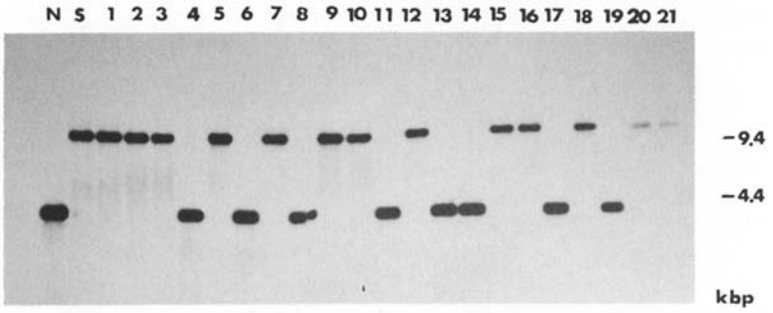
Mitochondrial DNA polymorphism

Samples of total mtDNA from N (W202B) and CMS-S (W202A) cytoplasms were cut with the *Bam*HI or *Hin*dIII enzyme, and the resulting fragments were separated by agarose gel electrophoresis. Characteristic and unique restriction profiles were exhibited by each mtDNA of the N and CMS-S type (Fig. 1), as previously observed (de Courcel et al. 1989; Holford et al. 1991).

In order to further study mtDNA organization in both cytoplasms, probes representing different mitochondrial genes were hybridized to membrane blots containg *Bam*HI or *Hin*dIII digests of the W202A and W202B mtDNAs. The genes used were: *rrn26,* coding for 26S ribosomal RNA; *cox-I* and *cox-II,* for cytochrome oxidase subunits I and II, respectively; *cob,* for apocytochrome B; and *atpA* and *atp9,* for ATPase subunits alpha and 9, respectively (see materials and methods). Figure 1 illustrates representative patterns of hybridization. For example, the *cob* probe hybridized to a *Bam*HI fragment of 4 kbp in W202B that was not present in W202A; instead in W202A this probe hybridized to an 11-kbp *Bam*HI fragment. Of the combinations of restriction enzyme and probe used here, only one, *Hin*dIII/*cob,* failed to distinguish the two cytoplasms (data not shown). These results are in agreement with those of Holford et al. (1991) except for the case of *Bam*HI/*cox-II*: those authors found no variation in the RFLP profiles generated with this combination of restriction enzyme and probe. It is thus apparent that the genomic surroundings of the six genes studied differ between N and CMS-S cytoplasms.

The variety 'Sapporo-ki'

Mitochondrial DNAs from 41 individual plants of this variety were tested by restriction enzyme analysis and Southern blot hybridization to see whether they were characteristic of normal onion (the W202B type) or of CMS-S onion (the W202A type). Hybridization of the *cob* probe to mtDNA digested with *Bam*HI revealed that 19 plants contained a 4-kbp W202B-specific fragment and the remaining 22 plants had an 11-kbp fragment characteristic of the W202A mtDNA (Fig. 2). This was further confirmed by *Bam*HI or *Hin*dIII restriction profiles and Southern blot analysis using the other five probes (data not shown). We have recently developed a group of CMS and maintainer lines from 'Sapporo-ki' (Y. Satoh and M. Nagai, unpublished data). A preliminary RFLP analysis of these lines demonstrated that the W202B type mtDNA is always associated with male fertility and that a similar correlation also exists for the W202A-type mtDNA and CMS (data not shown). Our data thus indicate that these mitochondrial gene probes allow the quick identification of N and CMS-S cytoplasms in the laboratory, though a genetic study of the 41 plants of 'Sapporo-ki' remains to be undertaken.



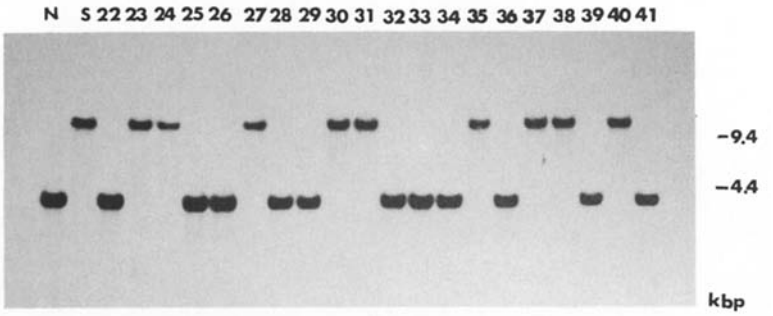


Fig. 2. Southern hybridization analysis of mtDNA from 41 individual plants of Japanese var 'Sapporo-ki'. The W202B(*N*) and W202A(*S*) mtDNA were included for comparison. MtDNA was cut with the *Bam*HI enzyme and electrophoresed in 0.8% agarose gel. Hybridization was done with the *cob* probe. Size markers are indicated in kbp

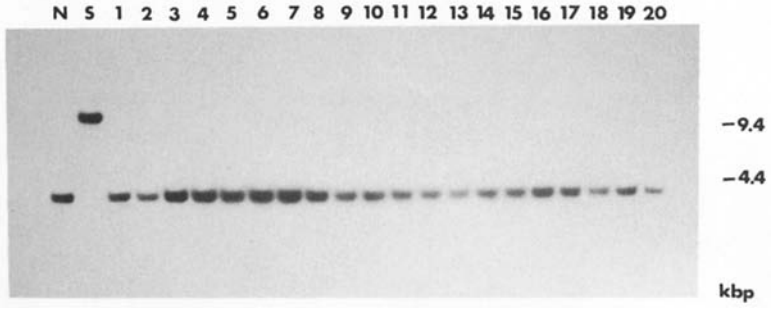


Fig. 3. Southern hybridization analysis of mtDNA from 20 individual plants of Japanese var 'Imai-wase'. The W202B(*N*) and W202A(*S*) mtDNA were included for comparison. MtDNA was cut with the *Bam*HI enzyme and electrophoresed in 0.8% agarose gel. Hybridization was done with the *cob* probe. Size markers are indicated in kbp

Also included in our study was a Japanese local variety 'Imai-wase' that is believed to be a selection from the old US variety 'Yellow Danvers' (T. Yakuwa, personal communication). As seen in Fig. 3, all of the 20 individual plants of 'Imai-wase' proved to have the W202B type mtDNA. 'Sapporo-ki' is, on the other hand, considered to be derived from another old US variety 'Yellow Globe Danvers', which was probably introduced into Japan in 1871 (T. Yakuwa, personal communication). It seems likely that 'Sapporo-ki' onion was never crossed with the Jones' CMS genotype by Japanese breeders. Intriguing questions remain unanswered about the origin of and the mechanism for maintenance of CMS within the population of 'Sapporo-ki' onion plants.

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