JARQ 28, 225-229 (1994)

Use of Mitochondrial DNA Polymorphism in the Classification of Individual Onion Plants by Cytoplasmic Genotypes

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Abstract

Individual plants of a Japanese onion variety Sapporo-ki, 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 presence of two classes of mtDNA variation within a population of Sapporo-ki: out of 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.

Discipline: Biotechnology/Horticulture
Additional key words: *Allium cepa*, cytoplasmic male sterility, RFLP

Introduction

Cytoplasmic male sterility (CMS) is widespread in plants and provides a convenient and appropriate means to produce hybrid seed[10]. In onion (*Allium cepa* L.), the original observation of CMS was reported by Jones[7] who found a male sterile plant in a population of the variety Italian Red. This material has given rise to nearly all the CMS lines presently used by breeders both in Japan and the United States.

The mitochondrial (mt) DNAs of the male fertile (normal) and the Jones' CMS onions were reported to give distinctive restriction profiles, respectively[2,5], thus allowing the rapid identification of a cytoplasm. If pairs of male sterile/maintainer lines were developed from locally adapted cultivars they could directly be used as seed parent in breeding programs[3]. A Japanese open-pollinated variety Sapporo-ki attracted our interest, because of the occasional occurrence of male sterile plants. The purpose of this study was to analyze the cytoplasmic genome variation in the onion

variety Sapporo-ki by restriction fragment length polymorphism (RFLP) analysis of mtDNA to facilitate the classification of individual plants by cytoplasmic genotypes.

Materials and methods

1) Plant materials

Analysis was carried out on individual plants of two Japanese open-pollinated varieties Sapporo-ki and Imai-wase from the onion germplasm collection preserved 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, USA.

2) Isolation of mtDNA

Mitochondria and mtDNA were isolated by a combination of differential centrifugation and DNase I-treatment according to Holford et al.[4].

Sprouting onion leaves (10 g) were homogenized in 40 m*l* of homogenization buffer (10 mM TES, 0.5 M mannitol, 10 mM EGTA, 0.2% BSA and 0.05% cysteine, pH7.2). The DNA obtained was purified by phenol-chloroform extraction, ethanol precipitation and RNase A-treatment.

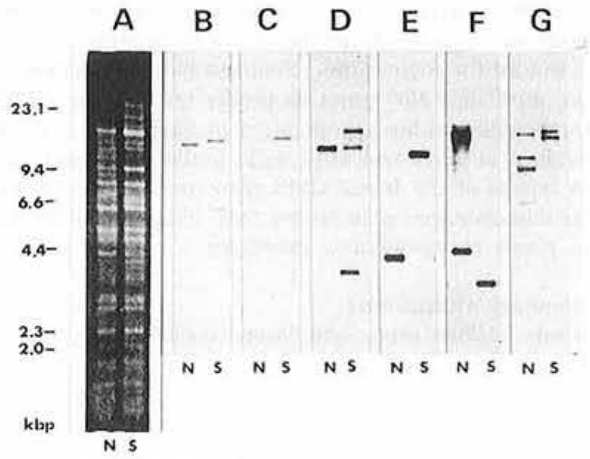

Plate 1. Southern hybridization analysis of mtDNA from W202B (N; N cytoplasm) and W202A (S; CMS-S cytoplasm)
MtDNA was cut with the *Bam*H I enzyme and electrophoresed in 0.8% agarose gel.
Panel A shows the restriction pattern after ethidium bromide staining of the gel.
The gel was blotted onto a nylon 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.

3) mtDNA analysis

Restriction endonuclease digestions were performed under conditions specified by the suppliers. The DNA was electrophoresed on agarose slab gels buffered with 40 mM Tris-HCl, 20 mM Na-acetate, 2 mM EDTA and 18 mM NaCl, pH8.0. The gels were Southern-blotted onto nylon membranes (Hybond N, Amersham) according to the manufacturer's instructions. 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. Then the hybridized blot was 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 with 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*R I -*Sal* I fragment[6]); sugarbeet *cox I* (1,500-bp *Eco*R I fragment[11]); sugarbeet *cox II* (400-bp *Sal* I -*Hin*d III fragment[11]) as well as wheat *cob* (700-bp *Hin*d III -*Eco*R I fragment[1]); pea *atpA* (800-bp *Eco*R I -*Bam*H I fragment[8]) and pea *atp9* (700-bp *Xho* I -*Eco*R V fragment[9]).

Results and discussion

1) mtDNA polymorphism

Samples of total mtDNA from N (W202B) and CMS-S (W202A) cytoplasms were cut with

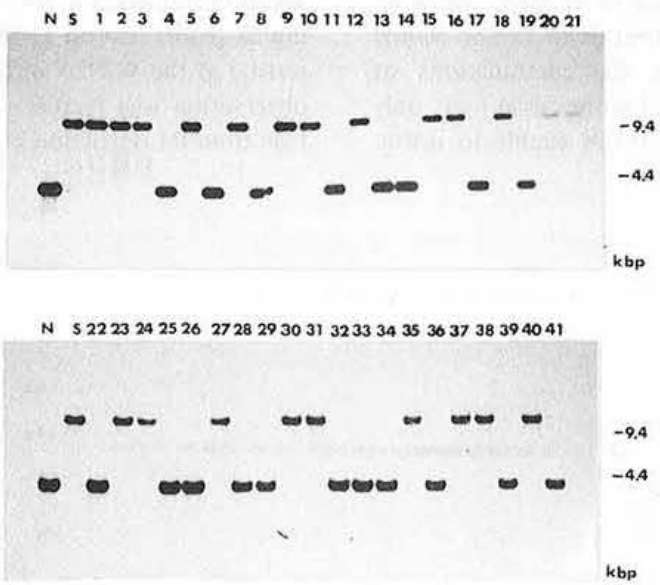

Plate 2. Southern hybridization analysis of mtDNA from 41 individual plants of a Japanese variety Sapporo-ki
The W202B (N) and W202A (S) mtDNA were included for comparison.
MtDNA was cut with the *Bam* H I enzyme and electrophoresed in 0.8% agarose gel.
Hybridization was performed with the *cob* probe.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

*Bam*H I or *Hin*d III 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 cytoplasms (Plate 1), which agreed well with previous observations[2,5].

In order to further study mtDNA organization in both cytoplasms, probes representing different mitochondrial genes were hybridized to membrane blots containing *Bam*H I or *Hin*d III 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; *cob*, for apocytochrome B; and *atpA* and *atp9*, for ATPase subunits alpha and 9 (see materials and methods). Plate 1 illustrates the representative patterns of hybridization. For example, the *cob* probe hybridized to a *Bam*H I fragment of 4-kbp in W202B, absent in W202A, which instead could be hybridized to an 11-kbp *Bam*H I fragment. Among the combinations of restriction enzymes and probes used here, only one, *Hin*d III/*cob*, did not enable to distinguish the two cytoplasms (data not shown). These results are in agreement with those of Holford et al.[5] except for the case of *Bam*H I/*cox II*, where they did not detect any variation in the RFLP profiles generated with this combination of restriction enzymes and probes. It is thus apparent that the genomic surroundings of the six genes studied differed between N and CMS-S cytoplasms.

2) The variety Sapporo-ki

Mitochondrial DNAs from 41 individual plants of this variety were tested by restriction enzyme analysis and Southern-blot hybridization to determine whether they were characteristic of normal onion (the W202B type), or CMS-S onion (the W202A type). Hybridization of the *cob* probe to mtDNA digested with *Bam*H I 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 (Plate 2). This observation was further confirmed by *Bam*H I or *Hin*d III restriction profiles and Southern-

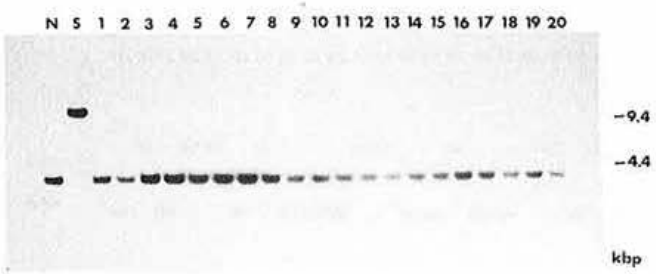

Plate 3. Southern hybridization analysis of mtDNA from 20 individual plants of a Japanese variety Imai-wase
The W202B (N) and W202A (S) mtDNAs were included for comparison.
MtDNA was cut with the *Bam*H I enzyme and electrophoresed in 0.8% agarose gel.
Hybridization was performed with the *cob* probe.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

blot analysis using the other five probes (data not shown). Recently two of the authors have developed a group of CMS and maintainer lines from Sapporo-ki (Y.Satoh and M.Nagai, unpublished). A preliminary RFLP analysis of these lines demonstrated that the W202B type mtDNA was always associated with male fertility and that a similar correlation also existed for the W202A type mtDNA and CMS (data not shown). Our data thus indicated the mitochondrial gene probes used to allow 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.

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

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(Received for publication, April 6, 1994)