SHORT PAPER

Classification of chloroplast DNA in *Brassica napus* possessing normal and male sterile cytoplasms

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ABSTRACT

Using restriction endonuclease analysis, chloroplast DNA (ctDNA) of normal or male sterile cytoplasms in *Brassica napus* were compared. There were some differences in restriction patterns of ctDNA from two types of cytoplasms digested with several restriction endonucleases. Based on the fragment pattern of Eco RI digested ctDNA, there were two types of ctDNAs in *B. napus* and one type of ctDNA was coincident with male sterile cytoplasm, whereas another type of ctDNA was observed only in the normal cytoplasm.

1. INTRODUCTION

Restriction endonuclease analysis of chloroplast DNA (ctDNA) provides a new information concerning the evolutionary relationship of *Brassica* species (Palmer *et al.* 1983, Erickson *et al.* 1983). In *B. napus*, two types of cytoplasms, namely normal or male sterile cytoplasm, have been reported (Thompson 1972, Shiga and Baba 1973). Recently, variations in ctDNA of *B. napus* and its relatives have been presented (Ichikawa and Hirai 1983, Li and Liu 1983). However, the number of breeding lines or cultivars subjected to ctDNA analysis by these workers is limited. Furthermore, the role of restorer genes in relation to ctDNA and male sterility has not been understood. The present study was carried out to evaluate the ctDNA restriction analysis as a means for the classification of *B. napus* plants possessing a normal or a male sterile cytoplasm.

2. MATERIALS AND METHODS

Seven male sterile lines from our breeding stocks, six Japanese cultivars with a male sterile cytoplasm and five cultivars with a normal cytoplasm were used for ctDNA analysis. A list of plant materials is summarised in Table 1, which indicates the type of cytoplasm and the presence of fertility restorer

genes (in nuclear). Plants were grown in a greenhouse.

Chloroplast DNA was isolated from leaves of two-month-old plants according to a modified method reported by Saltz and Beckman (1981). Leaves (about 50g) were homogenized with 250 ml buffer A [50 mM Tris-HCl (pH 8), 0.35M sucrose, 7mM EDTA-2Na, 5 mM 2-mercaptoethanol, 0.1% BSA] in a Waring blender, and filtered through 4 layers of cheese-cloth and 2 layers of Miracloth. The filtrate was centrifuged at $800\times g$ for 10 min, and the pellet was gently suspended in 10 ml buffer A, which was then laid on a stepwise sucrose gradient [20–45–60% sucrose, 50 mM Tris-HCl (pH 8), 0.3M sorbitol, 7 mM EDTA-2Na]. Following the centrifugation at $1,400\times g$ for 30 min, the green fraction (chloroplasts) was collected and mixed with 3 volumes of buffer B [50 mM Tris-HCl (pH 8), 20 mM EDTA-2Na] and centrifuged at $4,000\times g$ for 5 min. The chloroplast pellet was resuspended in 8 ml buffer B plus 2 ml 10% Sarcosyl, contained in a round bottom flask. After 20 min, 10 ml chloroform-phenol (1:1) was added to a flask, which was then rotated for 30 min. A clear supernatant was obtained by centrifugation ($1,500\times g$, 10 min), and the same chloroform-phenol extraction was repeated twice. Finally, to the clear supernatant was added by 1/10 volume of 3M Na-acetate and 2 volumes of cold ethanol. Chloroplast DNA was collected by a centrifugation ($4,000\times g$, 20 min) and dissolved in an appropriate buffer.

Chloroplast DNA was digested with the following enzymes: Eco RI, Bam HI, Hind III, Sal I, Sma I, Kpn I, Xho I, Pvu II and Bgl II (Takara Shuzo, Japan). Digested samples were electrophoresed in a 0.8% agarose gel containing 36 mM Tris-HCl (pH 8), 30 mM sodium mono phosphate and 1 mM EDTA-2 Na. Hind III digested fragments of lambda phage DNA were used as a molecular size marker.

3. RESULTS AND DISCUSSION

One of the objectives of this investigation was to compare restriction endonuclease digested ctDNAs in our male sterile breeding stock [(*chi*) Murasaki-natane] and the maintainer (Murasaki-natane). The floral organ of (*chi*) Murasaki-natane is characterised in the presence of small and rugose petals, of abnormal stamens with short filaments and poorly developed anthers without loculi nor pollen grains (Fig. 1). Among the restriction enzymes tested, Eco RI, Bam HI, Sma I and Kpn I digestions discriminated ctDNA of two lines (Fig. 2). In the case of Eco RI, (*chi*) Murasaki-natane possessed a 6.4 kbp fragment (Type I ctDNA), whereas Murasaki-natane had a 3.2 kbp fragment (Type II ctDNA).

Based on the criterion, other cultivars or lines containing different cytoplasms and restorer genes were investigated. The result summarised in Table 1 indicates that cultivars containing a normal cytoplasm had Type II ctDNA,

Fig. 1. Morphological characteristics of cytoplasmic male sterile [(*chi*) Murasaki-natane; a, b] and fertile [Murasaki-natane; c, d] flowers.

Fig. 2. Agarose gel electrophoretic patterns of ctDNA digested with Eco RI (a, b), Bam HI (c, d), Sma I (e, f) and Kpn I (g, h) restriction endonucleases. Lanes a, c, e and g, (*chi*) Murasaki-natane (male sterile); and b, d, f and h, Murasaki-natane (fertile). λ , lambda DNA markers of 23.7, 9.5, 6.7, 4.3 and 2.0 kbp from the top.

whereas plants with a male sterile cytoplasm had Type I ctDNA. The same ctDNA pattern was observed in four male sterile lines possessing the male sterile cytoplasm reported by Shiga and Baba (1973) and three male sterile lines possessing Thompson's (1972) ones (Table 1). Of particular interest was that nuclear restorer genes had no relation to ctDNA.

Table 1. *Type of ctDNA of 18 cultivars or lines in B. napus whose cytoplasmic type and fertility restorer genes are indicated*

Cultivar/line	Cytoplasmic type ^{a)}	Restorer gene ^{b)}	ct DNA type ^{c)}
Cytoplasmic male sterile lines			
MS line ^{d)}	S	rf	I
(<i>chi</i>) Isuzu-natane ^{d)}	S	rf	I
(<i>chi</i>) Murasaki-natane ^{d)}	S	rf	I
(<i>chi</i>) Bronowski ^{d)}	S	rf	I
(72/438/5 × Isuzu-natane) BC ₃ ^{e)}	S	rf	I
(72/891 × Isuzu-natane) BC ₁ ^{e)}	S	rf	I
(R. D. 15 × Bronowski) BC ₄ ^{e)}	S	rf	I
Cultivars with normal cytoplasm			
Isuzu-natane	N	rf	II
Murasaki-natane	N	rf	II
Bronowski	N	rf	II
Michinoku-natane	N	Rf	II
Miyuki-natane	N	Rf	II
Cultivars with male sterile cytoplasm			
Chisaya-natne	S	Rf	I
Asahi-natane	S	Rf	I
Kongo-natane	S	Rf	I
Mutsu-natane	S	Rf	I
Homan-natane	S	Rf	I
Iwashiro-natane	S	Rf	I

a) S: male sterile cytoplasm, N: normal cytoplasm

b) Rf: having fertility restorer genes, rf: no restorer gene

c) Type I and II are shown in Fig. 2 a and b, respectively.

d) Lines possessing a male sterile cytoplasm reported by Shiga and Baba (1973)

e) Lines possessing male sterile cytoplasm reported by Thompson (1972)

Type I (male sterile cytoplasm) and Type II (normal) ctDNA found in this work were identical to that of *B. oleracea* and *B. campestris* reported by Ichikawa and Hirai (1983), respectively. Therefore, studies on the role of ctDNA in the expression of male sterility using artificial *B. napus* (*B. oleracea* × *B. campestris*) are in progress. We do not have any informations about mitochondrial DNA yet. But from the data on Table 1, it was concluded that restriction endonuclease analysis of ctDNA appears to be a useful means of identifying male sterile cytoplasm in the breeding program of *B. napus*.

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REFERENCES

- ERICKSON, L. R., STRAUS, N. A. and BEVERSDORF, W. D. (1983) Restriction patterns reveal origins of chloroplast genomes in *Brassica* amphiploids. *Theor. Appl. Genet.* **65**, 201-206.
- ICHIKAWA, H. and HIRAI, A. (1983) Search for the female parent in the genesis of *Brassica napus* by chloroplast DNA restriction patterns. *Jpn. J. Genet.* **58**, 419-424.
- LI, J. and LIU, Y. (1983) Chloroplast DNA and cytoplasmic malesterility. *Theor. Appl. Genet.* **64**, 231-238.
- PALMER, J. D., SHIELDS, C. R., COHEN, D. B. and ORTON, T. J. (1983) Chloroplast DNA evolution and the origin of amphidiploid *Brassica* species. *Theor. Appl. Genet.* **65**, 181-189.
- SALTZ, Y. and BECKMAN, J. (1981) Chloroplast DNA preparation from *Petunia* and *Nicotiana*. *PMB Newslett.* **2**, 73-74.
- SHIGA, T. and BABA, S. (1973) (Cytoplasmic male sterility in oil seed rape, *Brassica napus* L., and its utilization to breeding) *Jpn. J. Breed.* **23**, 187-197.
- THOMPSON, K. F. (1972) Cytoplasmic male sterility in oilseed rape. *Heredity* **29**, 253-257.