Search for the female parent in the genesis of *Brassica napus* by chloroplast DNA restriction patterns

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ABSTRACT

Chloroplast DNAs (ctDNAs) from *Brassica* species were analyzed to identify the female parent of *B. napus*, which is the amphidiploid after the interspecific hybridization between *B. oleracea* and *B. campestris*.

The putative parents and their reciprocal hybrids were subjected to ctDNA analysis by *Bam* HI restriction patterns. The results demonstrated ctDNA from each of the reciprocal hybrids was identical to that from the female parent of the hybrids, showing the maternal inheritance of ctDNA in these particular crosses.

B. napus ctDNA was compared with the putative parental ctDNA by *Bam* HI and *Eco* RI restriction patterns. The results showed that *B. napus* ctDNA was indistinguishable with *B. oleracea* ctDNA and different from *B. campestris* ctDNA. From above evidences, we concluded that *B. oleracea* was the female parent of *B. napus*, although it seems to be very rare to arise the hybrids by cross between ♀ *B. oleracea* and ♂ *B. campestris* in nature.

1. INTRODUCTION

Plants belonging to the genus *Brassica* are economically important for agriculture and scientifically interesting by possessing well characterized chromosomes. The origin of *Brassica* species was, therefore, well studied mainly by cytogenetic analysis. Morinaga (1934) classified the cultivated species of *Brassica* into 6 groups, three elemental species and three amphidiploid species.

It is now established that *B. juncea*, *B. napus* and *B. carinata* are amphidiploids possessing genome AB, AC, and BC, respectively (Vaughan 1977). Fraction I protein, a genetic marker for nuclear and chloroplast informations (Wildman 1979), was analyzed to identify the female partner in putative parents (Uchimiya and Wildman 1978). *B. nigra* was identified as the female partner for *B. carinata*, and *B. campestris* for *B. juncea*. However, the female partner for *B. napus* was not identified by this method, since the large subunits of Fraction I protein from *B. oleracea* and *B. campestris* had the same isoelectric points.

To identify the female parent of *B. napus*, we have analyzed chloroplast

DNA (ctDNA) restriction patterns in *Brassica* species. In this communication, we show that ctDNA is transmitted to offspring through the female parent alone in the reciprocal crosses between *B. campestris* and *B. oleracea*, and demonstrate ctDNA in *B. napus* is indistinguishable with that in *B. oleracea*, thus *B. oleracea* is the female parent of *B. napus*.

2. MATERIALS AND METHODS

Plant materials

Seeds of cabbage (*B. oleracea*) and turnip (*B. campestris*) were purchased from a local store. Seeds of two kinds of rape (C-333: *B. campestris* and N-132: *B. napus*) and Swedish turnip (N-261: *B. napus*) were gifts from Dr. Takahata, Faculty of Education, Iwate University and Dr. Hinata, Faculty of Agriculture, Tohoku University, respectively. Plants were grown in a greenhouse. Cultivar of Chinese cabbage (*B. campestris*) was purchased at a local market. The reciprocal hybrid plants between *B. oleracea* and *B. campestris* were a gift from Dr. Takada, Gifu-ken Agricultural Experiment Station.

Isolation of chloroplast DNAs

Chloroplast DNAs were prepared according to Saltz and Beckman (1981) with modifications. Leaves were blended in a Waring blender with 7 volumes of buffer A (50 mM Tris-HCl, pH 8.0; 0.35 M sucrose; 7 mM EDTA; 5 mM β -mercaptoethanol) containing 0.1% BSA. The homogenate was filtered through four layers of gauze, then two layers of Miracloth, and centrifuged for 10 min at $1000\times g$. The pellet was washed once with buffer A. The green pellet suspended in buffer A was loaded on top of a stepwise 20–45–60% sucrose gradient made in 50 mM Tris-HCl, pH 8.0; 0.3 M sorbitol; 7 mM EDTA, and centrifuged for 30 min at $2000\times g$. The green band at the middle of the tube was collected, diluted 1:3 with buffer B (50 mM Tris-HCl, pH 8.0; 20 mM EDTA), and centrifuged for 10 min at $3000\times g$. The chloroplast pellet was resuspended in buffer B, and lysed by adding sodium dodecylsarcosinate at a final concentration of 3%. One twentieth volume of 10 mg/ml Pronase E (Kaken Kagaku Co. Ltd.) was added to the solution, and incubated overnight at 37°C. DNA was extracted from the lysate twice with phenol and once with phenol-chloroform (1:1). The DNA was precipitated with 2.5 volumes of ethanol from the aqueous phase. The precipitate was washed twice with 70% ethanol and dissolved in TE buffer (1 mM Tris-HCl, pH 8.0; 0.1 mM EDTA).

Restriction endonuclease analysis

Bam HI, *Eco* RI and *Sal* I were purchased from Takara Shuzo Co. Ltd.

and DNA was digested according to the supplier's instructions. Electrophoresis was performed on 0.7–1.5% agarose gels by the method of Sugiura and Kusuda (1979).

3. RESULTS AND DISCUSSION

Chloroplast DNAs were isolated from Chinese cabbage (*B. campestris*) and cabbage (*B. oleracea*), and digested by the restriction enzyme *Bam* HI. These DNA fragments were fractionated by agarose gel electrophoresis. As shown in Fig. 1, Chinese cabbage ctDNA is different from ctDNA from cabbage by the restriction patterns. Thus, it is possible to distinguish ctDNAs from Chinese cabbage and cabbage. We also analyzed ctDNAs from the reciprocal hybrids of the two species, two strains for which Chinese cabbage was the female parent and two strains cabbage as the female parent. The results showed ctDNA from each of the hybrids was identical to that from the female parent, showing that ctDNA is transferred from the female parent in the

Fig. 1 Agarose gel electrophoresis of *Bam* HI digest of ctDNAs from leaves of Chinese cabbage (1), ♀ Chinese cabbage × ♂ cabbage strain 54(2) and 55(3), ♀ cabbage × ♂ Chinese cabbage strain Hakuran (4) and Kouran (5), and cabbage (6). Lambda DNAs digested by *Bam* HI (7) and *Hind* III(8) were used as standards. Specific fragments for *B. campestris* (C) and *B. oleracea* (O) are shown between lane 3 and 4.

Fig. 2A Agarose gel electrophoresis of *Bam* *HI* digest of ctDNAs from leaves of *B. oleracea* (cabbage:1), *B. napus* (rape:2, Swedish turnip:3), and *B. campestris* (turnip:4, Chinese cabbage:5, rape:6). Lambda DNAs digested by *Bam* *HI* (7) and *Hind* *III* (8) were used as standards. Specific fragments are shown as in Fig. 1.

cross between *B. oleracea* and *B. campestris*.

Swedish turnip and rape in *B. napus* were subjected to ctDNA analysis by *Bam* *HI* and *Eco* *RI* restriction patterns as shown in Fig. 2, those DNAs are different from any DNAs from three varieties in *B. campestris*, whereas they are identical to *B. oleracea* ctDNA.

The restriction patterns by *Sal* *I* were also compared in *B. napus* and two putative parents, because Lebacqz and Vedel (1981) had reported that *Sal* *I* fragmentation patterns of *B. napus* and *B. oleracea* ctDNAs to be characteristically different on agarose gels. However, our results did not agree with their results, because ctDNAs from three species were indistinguishable by *Sal* *I* digestion as shown in Fig. 3.

U (1935) obtained the hybrid plants by ♀ *B. campestris* × ♂ *B. oleracea*, but could not by crossing *B. oleracea* as the female partner. Our results seem

Fig. 2B *Eco RI* digest of ctDNAs from *B. campestris* (Chinese cabbage:1, turnip:2, rape:3), *B. napus* (Swedish turnip:4, rape : 5), and *B. oleracea* (cabbage : 6). Specific fragments are shown as in Fig. 1.

to be discrepant with his evidence. The possibility that *B. campestris* was the female partner in the genesis of *B. napus*, but ctDNA was transferred from the pollen partner, was denied by our evidence (Fig. 1). We have, therefore, concluded from our restriction pattern analysis of ctDNA that *B. oleracea* was the female partner in the genesis of *B. napus*.

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Fig. 3 Agarose gel electrophoresis of *Sal I* digest of ctDNAs from leaves of *B. campestris* (rape:2, Chinese cabbage:3), *B. napus* (Swedish turnip:4, rape:5), and *B. oleracea* (cabbage:6). Lambda DNA digested by *Hind III* (1) was used as a standard.

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