Comparison of *B. napus* to *B. camepstris* and *B. oleracea* Based on the Cytoplasmic Characters; Cytoplasmic Male Sterility and Chloroplast DNA

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Brassica is one of the genera well studied regarding the relationship among the species in a genus. For the study, many methods, for instance, morphological, cytological, biometrical and biochemical analyses were used. Especially, by the genome analysis, it was found that 3 major monogenomic species, *B. campestris*, *B. nigra* and *B. oleracea*, participated in amphidiploid species, *B. juncea*, *B. carinata* and *B. napus* (Fig. 1).[12] These taxonomic studies were based on analyzing nuclear information only. Alternatively, a plant has another genetical information on the cytoplasm, maternally inherited characters. The cytoplasmic information is useful for finding out maternal ancestor of a species.

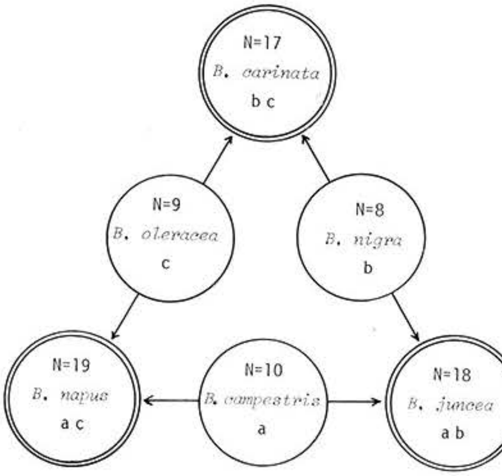

Fig. 1. Diagramatic representation of the genomic relations among the species in *Brassica* (modified)[12]

The present review shows the relationship of *B. napus* (aacc genome) to *B. campestris* (aa) and *B. oleracea* (cc) on the basis of cytoplasmic male sterility (CMS) and chloroplast DNA (ctDNA).

Cytoplasmic male sterility

Several kinds of CMS controlled by male sterile cytoplasm and restorer genes in nucleus were found in genus *Brassica* and its relatives.[4]

In *B. napus*, CMS was found out in F_2 progenies of intra-specific crosses.[8,10] The floral characteristics of the male sterile plants were 1) small and rugose petals, 2) abnormal stamens with short filaments and poorly developed anthers and 3) small anthers without pollen (Plate 1). The special feature of the CMS was that over 70% of cultivars had male sterile cytoplasm and fertility restorer gene(s), namely, it was concluded that the male sterile cytoplasm was the major cytoplasm in *B. napus* (Table 1).[6,9] Alternatively, only 3 maintainers were detected yet.

In *B. campestris*, it was found that a male sterile cytoplasm was present.[3] The male sterile cytoplasm was presumed to be in only 3 out of 56 cultivars/lines (Table 2). About 50% of cultivars had no restorer gene, i.e., there were many maintainers in *B. campestris*. The male sterile plants had distinguishable floral characters; small anthers

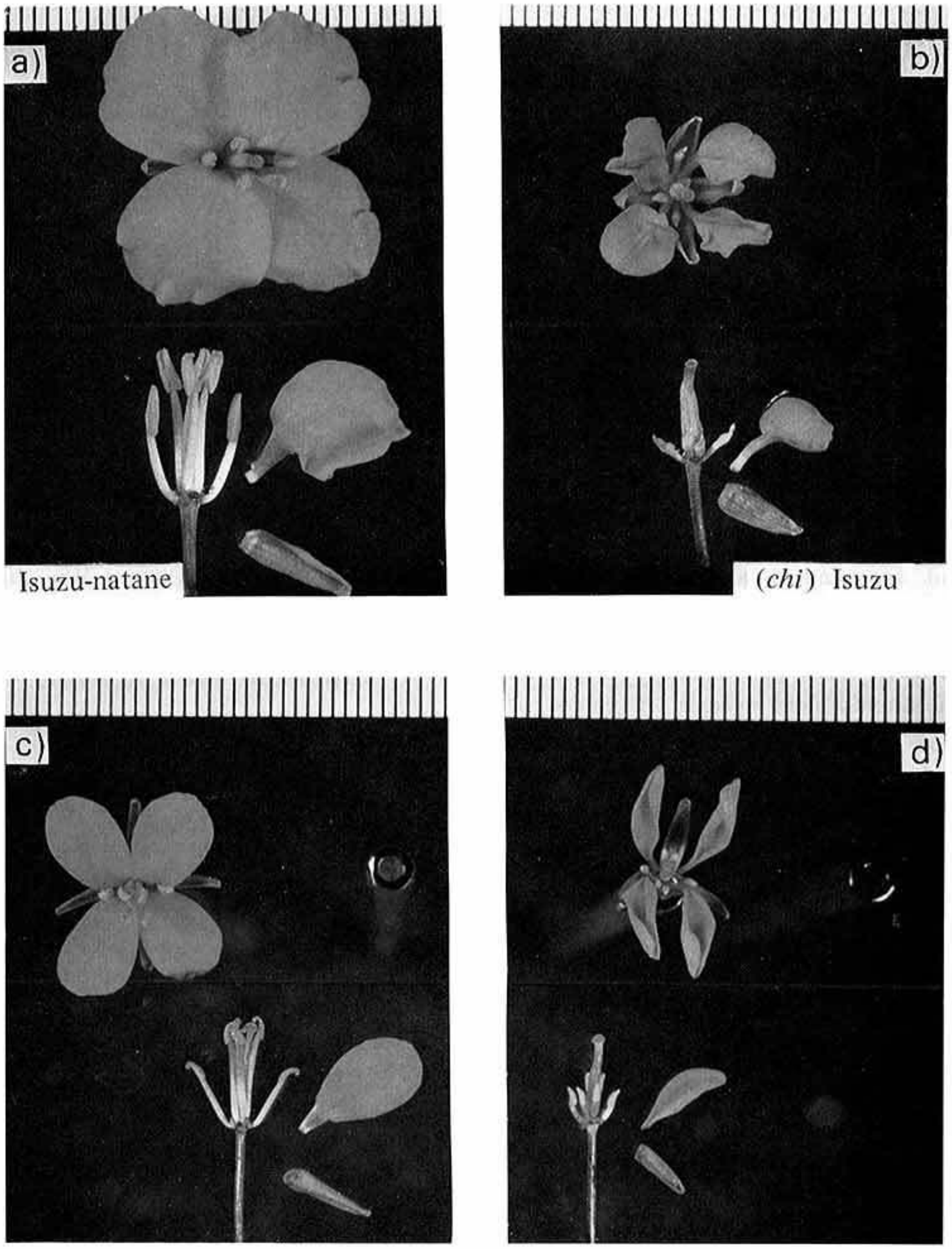

Plate 1. Male sterile and fertile flowers of *B. napus* and *B. campestris*
a) Maintainer of *B. napus*, b) CMS line of *B. napus*, c) Maintainer of *B. campestris*, d) CMS line of *B. campestris*.[3,8]

Table 1. Number of rapeseed (*B. napus*) varieties classified according to whether they have sterile or normal cytoplasm (modified)[6,9]

Variety/cytoplasm	Sterile	Normal	Indeterminalble	Total
Japanese	92	37		129
European	18	16	39	73
Total	110	53	39	202

without loculi nor pollen grains, short filament resulting in anthers having a lower position in relation to the stigma, and narrow petals (Plate 1) as mentioned in *B. napus*. The male sterility was manifested by a decrease in the number of pollen grains not by an increase of the ratio of sterile/normal pollen grains.

In *B. oleracea*, there were cytoplasmic male sterile lines but the sterile cytoplasm was derived from a male sterile line in *Raphanus*.

Table 2. Cytoplasmic type of cultivars or lines in *B. campestris*[4]

B. campestris ssp.	No. plants observed	Cytoplasmic type		
		Normal	Sterile	Indeterminable
Japanese varieties				
chinensis	3	3		
narinosa	1	1		
nipposinica	1	1		
oleifera	4	4		
parachinensis	1	1		
pekinensis	15	15		
rapifera	9	6		3
European varieties				
oleifera	8	1		7
rapifera	10	4	3	3
?	3	2		1
4n (*rapifera* × *chinensis*)	1			1
Total	56	38	3	15

Table 3. Degree of fertility of back-crossed populations[5]

Line/hybrid		No. plants observed	Relative position of anther/stigma[1]						
			1.0	2.0	3.0	4.0	5.0	6.0	Mean
I 4 (pms[2] in *B. campestris*)		10				2	7	1	4.90
(I 4 × Sensuji-kyona) BC_2 (do)		13	2	5	6				2.31
(I 4 × Wase-imaichi) BC_2 (do)		16	2	6	8				2.38
(I 4 × Isuzu-natane) BC_4	Mn[3]	15		12	3				2.20
(I 4 × Bronowski) BC_2	Mn	16		16					2.00
(I 4 × Mutsu-natane) BC_1	Rn	20					6	14	5.70
MS (cms[2] in *B. napus*)		53		5	39	8	1		3.09
(*chi*) Isuzu-natane (do)		59	43	14	2				1.31
(MS × Pakuchoi) BC_7	Mc	18	1	10	7				2.33
((*chi*) Isuzu-natane × Wase-imaichi) BC_1	Mc	8	4	3	1				1.63
((*chi*) Isuzu-natane × I 92) BC_3	Rc	20					2	18	5.90

1) 1 and 2 are male sterile, 3 and 4 are partially male sterile, and 5 and 6 are male fertile.
2) Pms and cms denote partially male sterile and male sterile, respectively.
3) Mn (or Mc): Maintainer for cms line in *B. napus* (or *B. campestris*)
Rn (or Rc): Restorer for cms line in *B. napus* (or *B. campestris*)

The cytoplasm of *B. oleracea* did not induce male sterility in *Brassica* species yet.[4]

As shown in Plate 1, the floral characteristics of male sterile *B. napus* were the same as male sterile *B. campestris*. On the other hand, by using crossing analysis, following results were obtained. The CMS in *B. campestris* was maintained by the maintainers for the CMS in *B. napus* (abbreviated to Mn) as well as by the maintainers for the CMS in *B. campestris* (abb. Mc) and restored by the restorer for the CMS in *B. napus* (abb. Rn) as well as by the restorers for the CMS in *B. campestris* (abb. Rc). Similarly the CMS in *B. napus* was maintained or restored by Mc or Rc as well as Mn or Rn, respectively (Table 3). From these results, as far as CMS is concerned, the male sterile cytoplasm and the normal cytoplasm of *B. napus* were assumed to be the same as those of *B. campestris*, respectively.

The cytoplasm of *B. oleracea* did not induce male sterility in the hybrids between *B. napus* and artificially synthesized *B. napus* bearing the cytoplasm of *B. oleracea* (Plate 2). On the contrary, the male sterile cytoplasm of *B. napus* induced male sterility in the hybrids. Accordingly, it was assumed that the cytoplasm of *B. oleracea* was similar to the normal cytoplasm of *B. napus* and almost *B. oleracea* cultivars had no restorer gene.[4]

Restriction endonuclease digested chloroplast DNA

Molecular biological studies on ctDNAs in *B. napus* consisting of inducing fragmentation by digestion with several endonucleases revealed that there were 2 types of patterns (Type I and II) in digested fragments of ctDNA with Eco RI restriction endonuclease (Fig. 2). Interestingly, cultivars containing a normal cytoplasm had Type II ctDNA, whereas plants with a male sterile cytoplasm

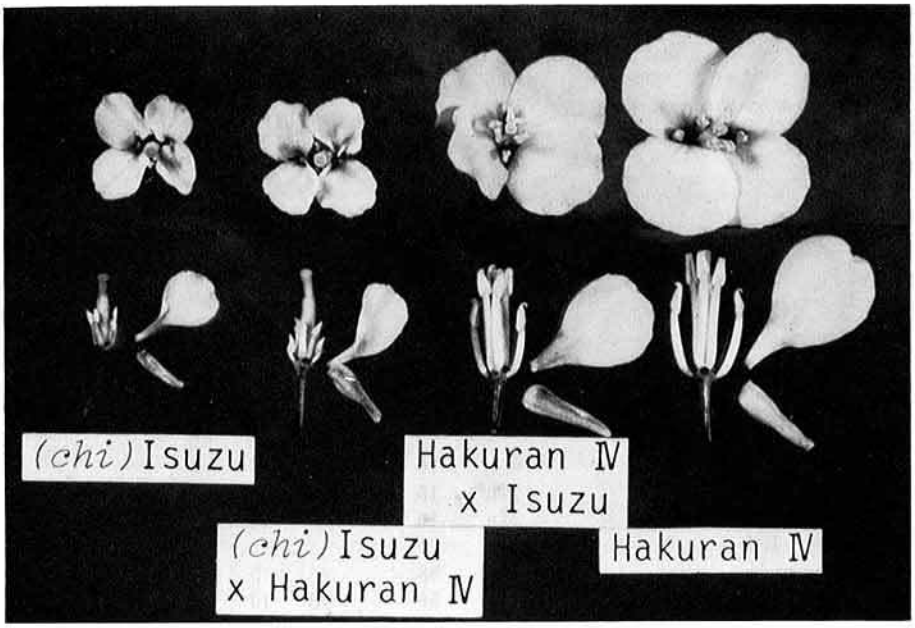

Plate 2. Degree of male sterility of hybrids between cytoplasmic male sterile line in *B. napus* and artificial *B. napus* (cc×aa)

(*chi*) Isuzu (CMS line of *B. napus*) and F_1 ((*chi*) Isuzu (♀)× Hakuran IV (♂)) had male sterile flowers, on the contrary, Hakuran IV harbouring the cytoplasm of *B. oleracea* and the reciprocal F_1 (Hakuran IV(♀)×Isuzu (maintainer of *B. napus*) (♂)) had normal flowers.

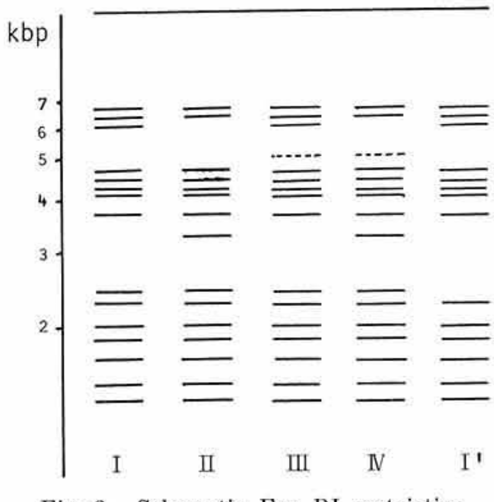

Fig. 2. Schematic Eco RI restriction fragment patterns of ctDNA from *B. napus* (I, II), *B. campestris* (III, IV) and artificial *B. napus* (I′) harbouring the cytoplasm of *B. oleracea*. (modified)[4]

had Type I ctDNA.[5] The same situation developed in *B. campestris*. There were 2 types of ctDNA, one consisted in the male sterile cytoplasm and the other in normal cytoplasm. All of the artificially synthesized *B. napus* harbouring the cytoplasms of *B. oleracea* which were used for the crossing experiments on CMS had only one type of ctDNA (Unpublished data).

Fig. 2 shows a comparative fragment pattern of ctDNA of *B. napus*, *B. campestris* and *B. oleracea* (artificially synthesized *B. napus*). There were slight differences between Type I, Type III and Type I′, and Type II and Type IV. The results of restriction endonuclease analysis of interspecific nuclear substituted plants indicated that Type I and Type II ctDNA were the same as Type III and Type IV, respectively, but Type I′ was different from Type I.[4]

Concluding remarks

According to many works of genomic analysis in genus *Brassica*, *B. napus* (aacc genome) was thought to originate from an interspecific hybrid between *B. campestris* (aa) and *B. oleracea* (cc). But it has not been determined which species was maternal ancestor. A key to solve the problem is in genetical analysis for cytoplasmically inherited characters and cytoplasmic DNA itself (chloroplast DNA and mitochondria DNA). An attempt will be made to discuss the relationship of these 3 species based on the results obtained in this review.

The male sterile cytoplasm and normal cytoplasm of *B. napus* were the same as those cytoplasms of *B. campestris*, respectively. In *B. napus*, the male sterile cytoplasm was major and normal cytoplasm was minor, but in *B. campestris*, most of all cultivars had normal cytoplasm. *B. napus* easily crossed with *B. campestris* and the reverse was true.[2] Furthermore, the *B. campestris* lines detected to have male sterile cytoplasm had not been grown in the cradle of *Brassica* species. Then, the male sterile cytoplasm of *B. campestris* is presumed to have come from *B. napus* by repeated backcrossing of *B. campestris* to *B. napus* plant harbouring male sterile cytoplasm. Some Japanese cultivars of *B. napus* possessing normal cytoplasm were pointed out to have come from interspecific hybrids, *B. campestris* (♀)×*B. napus* (♂).[7] In Europe, only *B. campestris* was cultivated for obtaining oil before the 16th century and was gradually replaced by *B. napus*.[11] It seems that there were some chances for interspecific crossing. Then the normal cytoplasm of *B. napus* is conceivable to be derived from *B. campestris*. Presumption mentioned above were supported by the results of restriction endonuclease analysis of ctDNA.

The major pattern of restriction endonuclease digested ctDNA of *B. napus* (Type I) which possessed male sterile cytoplasm was more similar to that of *B. oleracea* (Type I′) than *B. campestris* (Type IV or II). This was also reported by Ichikawa and Hirai.[1]

From the results of the study on CMS, the cytoplasms of both *B. oleracea* and *B. campestris* did not induce male sterility, and alternatively the cytoplasm of *B. napus* (sterile cytoplasm) induced male sterility. That is to say, the cytoplasm of *B. napus* was different from those of *B. oleracea* and *B. campestris*, although *B. napus* has been known to originate from a hybrid between *B. campestris* and *B. oleracea*. On the other hand, the data of the restriction endonuclease analysis on ctDNA indicated that the ctDNA of *B. napus* (Type I) was more similar to that of *B. oleracea* (Type I′) than that of *B. campestris* (Type II or IV). Preliminary data from our materials (unpublished) suggested that 7 out of 17 cultivars of *B. napus* harbouring normal cytoplasm did not have Type II but had Type I ctDNA. In conclusion, it was presumed that *B. napus* originated from the interspecific hybrid whose maternal parent was *B. oleracea* and paternal one was *B. campestris*.

References

1) Ichikawa, H. & Hirai, A.: Search for the female parent in the genesis of *Brassica napus* by chloroplast DNA restriction patterns. *Jpn. J. Genet.*, 58, 419–424 (1983).
2) Namai, H., Sarashima, M. & Hosoda, T.: Interspecific and intergeneric hybridization breeding in Japan. *In Brassica* crops and wild allies. Tsunoda, S., K. Hinata and C. Gomez-campo, ed. Japan Scientific Societies Press, Tokyo, 191–203 (1980).
3) Ohkawa, Y.: Cytoplasmic male sterility in *Brassica campestris* ssp. *rapifera* L. *Jpn. J. Breed.*, 34, 285–294 (1984).
4) Ohkawa, Y.: New cytoplasmic male sterility in *Brassica campestris* ssp. *rapifera* (Metzg.) Sinsk. and its relationship to the cytoplasmic male sterility in *B. napus* L. *Bull. Nat. Inst. Agr. Sci.*, Ser. D **36**, 1–55 (1985) [In Japanese with English summary].
5) Ohkawa, Y. & Uchimiya, H.: Classification of chloroplast DNA in *Brassica napus* possessing normal and male sterile cytoplasms. *Jpn. J. Genet.*, **60**, 249–253 (1985).
6) Shiga, T.: Studies on heterosis breeding using cytoplasmic male sterility in rapeseed, *Brassica napus* L. *Bull. Nat. Inst. Agr. Sci.*, D **27**, 1–101 (1976) [In Japanese with English summary].
7) Shiga, T.: Cytoplasmic-genetic male sterility and differentiation of cytoplasm in *Brassica* plant. *Recent Advance of Breeding*, **20**, 55–62 (1979) [In Japanese].
8) Shiga, T. & Baba, S.: Cytoplasmic male sterility in oil seed rape, *Brassica napus* L. and its utilization to breeding. *Jpn. J. Breed.*, **23**, 187–197 (1973) [In Japanese with English summary].
9) Shiga, T., Ohkawa, Y. & Takayanagi, K.: Cytoplasm types of European rapeseed (*Brassica napus* L.) cultivars and their ability to restore fertility in cytoplasmic male sterile lines. *Bull. Nat. Inst. Agr. Sci.*, Ser. D **35**, 103–124 (1983).
10) Thompson, K. F.: Cytoplasmic male sterility in oilseed rape. *Heredity*, **29**, 253–257 (1972).
11) Toxopeus, H.: The domestication of *Brassica* in Europe—Evidence from the herbal books of the 16th and 17th centuries. *In* Proceedings of a EUCARPIA-conference on the breeding of Cruciferous crops. compiled by N.P.A. van Marrewijk and H. Toxopeus, Wageningen, The Netherlands. 47–56 (1979).
12) U, N.: Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Jpn. J. Bot.*, **7**, 389–452 (1935).

(Received for publication, August 14, 1985)