

ORIGIN OF MALE STERILITY-INDUCING CYTOPLASM IN RAPE PLANT (BRASSICA NAPUS L.)

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Thompson (1972) and Shiga and Baba (1973) described independently the occurrence of cytoplasmic male sterility (CMS) in rape plant, Brassica napus, in which CMS had never been reported. Using this male sterile line (abbreviated to MS line) as a tester, Shiga (1976) classified Japanese cultivars of rape plant into nine groups, i.e. S-0, S-I, S-IIa, S-IIb, S-III, S-IV, N-0, N-I and N-II in which S designated line with the male sterility-inducing cytoplasm, N those with normal cytoplasm, and 0 to IV indicated the number of fertility restoring gene (s). Most of the Japanese rape cultivars had S cytoplasm but the remainder had N cytoplasm (Shiga, 1976). Thompson (1972) had also observed that Canadian and Swedish rape cultivars had S cytoplasm, while Polish spring rape cultivars (Bronowski) had N cytoplasm.

These results make it difficult to elucidate the origin of the S cytoplasm, which is so common in rape plant that mutation cannot be proposed as mechanism, unlike in other species. B.napus which has been considered an amphidiploid ($2n=38$, AACC genome) derives from natural crossing between species with ten ($2n=20$, AA) and nine ($2n=18$, CC) chromosomes (Morinaga, 1934). The present investigation aimed at determining from which species, AA or CC genome species, the S cytoplasm of B.napus derived under natural conditions. In this report we outline the results of artificial crossing between AA, AACC and CCAA genome species and MS line reciprocally, and so far conclude that N cytoplasm originate from AA genome species.

MATERIALS AND METHODS

The male sterile line used in the experiment was reproduced by selfing or sib-crossing with a few normal pollen grains formed under natural conditions. The higher the temperature was, the more normal pollen grains formed. For detecting the nature of cytoplasm, the MS line was reciprocally crossed with cultivars of AA genome species, B.chinensis, B.narinosa, B.parachinensis, B.pekinensis and B.rapa and artificially synthesized B.napus (AA X CC, abbreviated to CO). MS line was also crossed with the F_1 -hybrid plants of artificially synthesized B.napus (CC X AA, designated Kyabusai) and Murasaki-natane (B.napus, N-0) without restoring gene with normal cytoplasm.

The extent of male sterility in those hybrids was determined by the relative position of anther to stigma (Shiga, 1976). Among these, categories 1 and 2 were male sterile bearing short stamens with undeveloped anther, and normal pistil and stigma. Categories 3 and 4 were partially male sterile bearing shorter stamens with abnormal anther, and normal pistil and stigma. Categories 5 and 6 were almost normal male fertile (Fig. 1). The criterion of male sterility in this experiment was limited to morphological characteristics, because the materials used were inter-specific F_1 hybrids.

RESULTS AND DISCUSSION

The number of plants belonging to each category of male sterility in reciprocal F_1 hybrids resulting from crosses between MS line and AA genome species 1 (cultivars) in the 1976-1977 season are shown in Table 1. All F_1 hybrids between MS line and AA genome cultivars except for MS X Murasaki-daimaru-kabu were partially male sterile, while the reciprocals were completely male fertile.

Five F_1 hybrids out of six obtained from crosses between MS line and artificially synthesized B. napus (AACC, CO) grown in the 1976-1977 season were partially male sterile (Table 2). F_1 hybrids of artificially synthesized B. napus, Kyabusai (CCAA), and Murasaki-natane (N-O) were crossed with MS line reciprocally and both of the reciprocal hybrids were partially male sterile (Table 2).

From these experimental results we considered that thirteen out of fourteen AA genome cultivars examined had N cytoplasm with or without weak restoring genes except for Murasaki-daimaru-kabu which is a forage turnip rape growing in Japan. We assumed that natural crossing had occurred between turnip rape and rape (B. napus) in the past and that turnip rape had the chance of acquiring fertility-restoring genes from rape plant. Possible examples of genotypes and phenotypes in the reciprocal F_1 hybrids between MS line and AA genome species are shown below:

(MS X AA genome species)	--->	F_1 genotype	F_1 phenotype
1. (S)rfrf X (N)rfrf		(S)rfrf	male sterile
2. (S)rfrf X (N)RFRf		(S)Rfrf	(partially male sterile or male fertile)
(AA genome species X MS)	--->	F_1 genotype	F_1 phenotype
1. (N)rfrf X (S)rfrf		(N)rfrf	fertile
2. (N)RFRf X (S)rfrf		(N)Rfrf	fertile
3. (S)RFRf X (S)rfrf		(S)Rfrf	(fertile or partially male sterile)

In the case of artificially synthesized B. napus (AACC, CO) the results were similar to those obtained in AA genome species. CO were artificially synthesized amphidiploid originally crossed with AA genome species as female. It could not be determined whether CO-45 had N cytoplasm or not, but CO-45 was bred from progenies of cross between Lembke turnip rape and kale at Svalöv. Thus the possibility of the introduction of restoring genes from turnip rape or kale could be considered in the case of CO-45.

Another amphidiploid B. napus (CCAA, Kyabusai) is a new crop derived from crossing between cabbage (CC) and chinese cabbage (AA) performed by Nishi et al. (1957). Test crossing with MS line suggested that Kyabusai had S cytoplasm which could possibly originate from CC genome species. As the data on the cytoplasmic characteristics of CC genome species were not conclusive, it could not be established that S cytoplasm of B. napus originated from CC genome species. However, it could be demonstrated that AA genome species of Asia possessed N cytoplasm. To confirm the present findings a larger number of cultivars with AA and CC genome from Europe and India shall be used.

TABLE 1.

EXPRESSION OF MALE STERILITY IN HYBRIDS FROM CROSSES BETWEEN MS LINE OF BRASSICA NAPUS AND AA GENOME SPECIES, AND THEIR PARENTS

Parents and hybrids	Number of plants with relative position of anther to stigma						mean
	1	2	3	4	5	6	
<i>Brassica chinensis</i>							
Pakuchoi	-	-	-	1	3	2	5.70
MS X Pakuchoi	1	6	7	1	-	-	2.57
Pakuchoi X MS	-	-	-	-	14	1	4.96
Shigatsu-shirona	-	-	-	-	1	8	5.83
MS X Shigatsu-shirona	-	-	11	4	-	-	3.24
Shigatsu-shirona X MS	-	-	-	-	10	4	5.29
Yukishiro-taisai	-	-	-	-	9	2	5.29
MS X Yukishiro-taisai	1	6	7	1	-	-	2.40
Yukishiro-taisai X MS	-	-	-	-	14	-	4.86
<i>Brassica narinosa</i>							
Vitamin-na	-	-	-	-	3	9	5.63
MS X Vitamin-na	-	6	9	-	-	-	2.46
Vitamin-na X MS	-	-	1	1	12	1	4.80
<i>Brassica parachinensis</i>							
Tsaishin	-	-	-	1	3	-	4.78
MS X Tsaishin	-	1	12	1	-	-	2.75
Tsaishin X MS	-	-	-	-	10	3	5.16
<i>Brassica pekinensis</i>							
Maruba-santosai	-	-	-	-	2	13	5.77
MS X Maruba-santosai	3	4	7	1	-	-	2.26
Maruba-santosai X MS	-	-	-	1	13	1	5.00
Hikoshima-shunsai	-	-	-	-	8	4	5.33
MS X Hikoshima-shun.	-	-	11	4	-	-	3.20
Hikoshima-shun. X MS	-	-	-	1	10	4	5.20
Chirimen-hakusai	-	-	-	-	2	6	5.37
MS X Chirimen-haku.	-	6	9	-	-	-	2.26
Chirimen-haku. X MS	-	-	-	1	14	-	4.96
Kashin-hakusai	-	-	-	-	-	15	6.00
MS X Kashin-hakusai	1	6	8	-	-	-	2.27
Kashin-hakusai	-	-	-	1	5	9	5.33
Hakusai II	-	-	-	-	-	13	6.00
MS X Hakusai II	-	1	10	4	-	-	3.10
Hakusai II X MS	-	-	-	-	2	12	5.59
Hakusai VI	-	-	-	-	-	15	5.87
MS X Hakusai VI	1	-	12	2	-	-	3.07
Hakusai VI X MS	-	-	-	-	4	11	5.60
Hakusai XIII	-	-	-	-	2	12	5.79
MS X Hakusai XIII	-	1	9	5	-	-	3.20
Hakusai XIII X MS	-	-	1	2	7	5	5.04
<i>Brassica rapa</i>							
Murasaki-daimaru-kabu	-	-	-	-	6	1	5.07
MS X Murasaki-daimaru.	-	-	-	3	11	-	4.57
Murasaki-daimaru. X MS	-	-	-	-	14	-	5.07
Kanamachi-kokabu	-	-	-	-	2	1	5.34
MS X Kanamachi-kokabu	-	2	9	3	1	-	2.93
Kanamachi-kokabu X MS	-	-	1	2	12	-	4.70
MS (in 1976)	6	9	-	-	-	-	2.51

TABLE 2.

EXPRESSION OF MALE STERILITY IN HYBRIDS BETWEEN MS LINE OF B.NAPUS AND ARTIFICIAL B.NAPUS, AND THEIR PARENTS

Parents and hybrids	Number of plants with relative position of anther to stigma						mean
	1	2	3	4	5	6	
<u>Artificial B.napus (AACC)</u>							
CO-3	-	-	-	-	3	7	5.50
MS X CO-3	1	-	7	4	2	-	3.36
CO-3 X MS	-	-	-	-	2	13	5.50
CO-4	-	-	-	-	6	9	5.54
MS X CO-4	-	8	6	1	-	-	2.46
CO-4 X MS	-	-	1	2	4	4	5.00
CO-6	-	-	-	-	2	12	5.54
MS X CO-6	-	-	7	3	5	-	3.80
CO-6 X MS	-	-	-	-	4	11	5.57
CO-11	-	-	-	-	5	10	5.60
MS X CO-11	-	7	3	2	3	-	3.04
CO-11 X MS	-	-	-	-	-	15	5.64
CO-16	-	-	-	-	-	15	5.64
MS X CO-16	-	6	8	1	-	-	2.54
CO-16 X MS	-	-	-	-	12	3	5.24
CO-45	-	-	-	-	-	15	5.93
MS X CO-45	-	-	-	-	-	13	5.62
CO-45 X MS	-	-	-	-	-	14	5.83
<u>Artificial B.napus (CCAA)</u>							
Kyabusai	-	-	5	4	2	4	4.30
MS X (Kyabusai X Murasaki*)	-	7	7	1	-	-	2.50
(Kyabusai X Murasaki) X MS	-	1	6	3	3	2	3.84
(Kyabusai X Murasaki) F ₂	-	1	4	6	2	1	3.82
MS (in 1976)	6	9	-	-	-	-	2.51

*; Murasaki-natane has N cytoplasm and no restoring gene.



FIG. 1

PHENOTYPIC CRITERION OF MALE STERILITY

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