Cytoplasmic Male Sterility and Its Utilization for Heterosis Breeding in Rapeseed, *Brassica napus* L.

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Heterosis breeding of rapeseed, *Brassica napus* L., seems promising in providing stability and high productivity for rapeseed production. However, heterosis breeding has not been practiced in rapeseed, because it has been impossible to produce hybrid seeds economically.

Recently, Thompson (1972) reported a cytoplasmic male sterility found in F_2 generation from varietal rapeseed crosses. The present author and co-workers also found a cytoplasmic male sterile line in the progenies of varietal cross of Japanese rapeseed (Shiga and Baba 1971, 1973).

Occurrence and characteristics of this male sterile line will be described in this paper. Test crossing of many rapeseed cultivars with this male sterile line and progeny test were conducted, and cultivars were classified into 9 classes according to their cytoplasmic differences and their fertility restorer gene(s). It was proved that this cytoplasmic male sterility could be maintained and also the fertility could be restored by crossing this male sterile line with appropriate cultivars.

Extent of the occurrence of heterosis in intraspecific hybrids in *Brassica napus* will also be given, and finally the possibility of using the cytoplasmic male sterility in heterosis breeding will be discussed.

Rapeseed cytoplasmic male sterility

1) *Cytoplasmic male sterility derivation*

In spring 1964, at the Rapeseed Breeding Center, Fukushima Agricultural Experimental Station, the author selected 60 plants and bagged them for selfing in F_2 generation from an intraspecific cross between cultivars, Chisaya-natane as a female, and Hokuriku 23 as a pollen parent. These plants showed extremely low self fertility, and selection for complete male sterility was carried out in successive generations.

In spring 1970, it was confirmed that the cytoplasmic factor controls this male sterility, using the reciprocal crosses of Chisaya-natane and Hokuriku 23.

2) *Morphological observations*

Compared to plants of Chisaya-natane, the male sterile plants were slightly shorter, having nearly the same number of primary branches and somewhat lower seed fertility. The completely male sterile plants had the following distinctive floral characteristics: light colored and slightly small buds, small and rougose petals, abnormal stamens with short filaments and poorly developed anthers —these anthers were small elliptical cones without pollen grains. The partially male sterile plants had various-sized buds, petals and stamens, depending on the degree of male sterility. The plants can be classified into six types according to the degree of male sterility using the criteria shown in Plate 1; types 1 and 2 are male sterile, 3 and 4 are partially male sterile and 5 and 6 are normally male fertile.

3) *Cyto-histological observations*

Microscopic observations of transverse section of flower bud indicated that calyxes, petals and pistils of the male sterile plants developed rather normally, but, no anther produced four loculi and neither pollen mother cells nor anther tapeta were developed. These observations suggested that the inhibition of anther growth occurred between the stages of carpel differentiation III and male archesporial cell differentiation I. Anthers of partially male sterile plant produced male archesporial cells which sometimes developed into anther loculi. These loculi produced pollen mother cells and anther tapeta. The pollen mother cells grew into pollen grains through meiotic division and tetrad stages.

4) Male sterility maintenance

The male sterile plants were repeatedly back-crossed to the original male parent (Hokuriku 23) and it was confirmed over the following four generations that the male sterility was maintained. The male sterile plants sometimes produced functional pollen grains in the late flowering stage. The original male sterile line could be maintained by self-pollinating these pollen grains. When the progenies were maintained by such selfing with rarely formed pollen grains and were selected for the direction of complete male sterility, the proportion of completely male sterile plants increased.

5) F_1 seed production of male sterile lines

The seed set on the male sterile plants was fairly high both in the open pollinated condition and in the isolation field with a pollinator cultivar. Seed setting by bagging in the male sterile plants was negligible.

Fertility restoration

1) Searching for cultivars having fertility restoring ability

Searching for cultivars having fertility restoring ability to male sterile cytoplasm, 131 Japanese bred cultivars were crossed to the male sterile line. Floral characters in F_1 population were examined and correlation coefficients, partial correlation coefficients and multiple correlation coefficients among them were calculated. For the estimation of fertility restoring ability, the fertility index proposed by the author was used. This index was calculated from the values expressing relative position of anther to stigma and petal width by an equation (1).

Fertility Index (FI)

$$= (X_1 - C_1) B_1 + (X_3 - C_3) B_3 \ldots\ldots (1)$$

where

X_1: measured value of relative position of anther to stigma,

X_3: measured value of petal width,

C_1: value of relative position of anther to stigma in male sterile line,

C_3: value of petal width in male sterile line,

B_1 and B_3: constant.

Thus the Japanese cultivars were classified into three groups by the fertility indices of hybrids i.e., 23 fertility restoring cultivars, 79 partially-restoring cultivars and 29 non-restoring cultivars.

2) Fertility restorer gene(s)

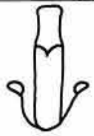
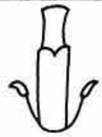
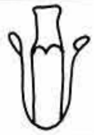
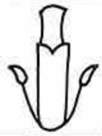
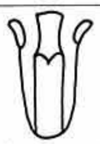
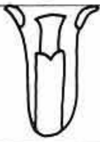
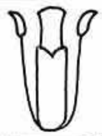
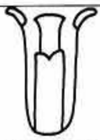
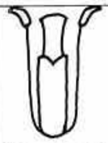
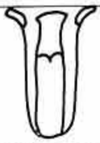
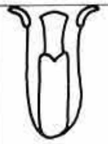
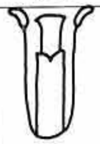
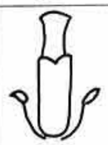
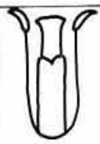
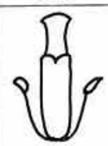
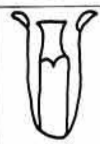
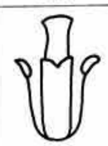
The fertility restorer gene(s) determines seed setting of hybrid between the male sterile line and the cultivars, and also determines the relative position of anther to stigma. Anther length and petal width are supposed to be controlled to a large extent either by pleiotropy of the fertility restorer gene(s) or the gene(s) linked closely to the fertility restorer gene(s). A portion of these two characters may be determined by other genetic mechanism.

3) Classification of rapeseed cultivars based on the cytoplasm and fertility restorer gene(s) differences

Frequency distribution of the relative position of anther to stigma and petal width in parental, F_2 and three back-cross populations from the crosses between male sterile line and the 14 Japanese cultivars was analyzed.

Table 1 shows the classification of rapeseed cultivars based on cytoplasmic differences and the number of the restorer genes. The phenotypes of reproductive organ in cultivars and hybrids of male sterile line with cultivars are

Table 1. Classification of rapeseed cultivars based upon the differences of cytoplasm and fertility restorer gene(s), and phenotypes of reproductive organ in cultivar and F_1 of MS × cultivar

Class	Cytoplasm	Fertility restorer	Relative position of anther to stigma: Cultivar		Relative position of anther to stigma: F_1 (MS X Cultivar)		Cultivar
S–0	S*	recessive gene		male sterile		male sterile	male sterile line
S–I	S	single incomplete dominant gene		partially male sterile		partially male sterile	Tokai 3 Chisayanatane
S–IIa	S	two incomplete dominant genes		male fertile		partially male sterile	Asahinatane Norin 16 Kongounatane
S–IIb	S	two incomplete & complete dominant genes		male fertile		male fertile	Hokuriku 7 Hokuriku 9
S–III	S	three dominant*** genes		male fertile		fully male fertile	Aomori 1
S–IV	S	four dominant*** genes		male fertile		fully male fertile	Mutsunatane
N–0	N**	recessive gene		male fertile		male sterile	Isuzunatane Murasakinatane
N–I	N	single incomplete dominant gene		male fertile		partially male sterile	Michinokunatane
N–II	N	two incomplete dominant genes		male fertile		partially male sterile	Miyukinatane? Norin 11?

* : S cytoplasm shows the male sterile-inducing cytoplasm.
** : N cytoplasm shows the normal (non-male sterile-inducing) cytoplasm.
*** : Complete or incomplete dominant gene.

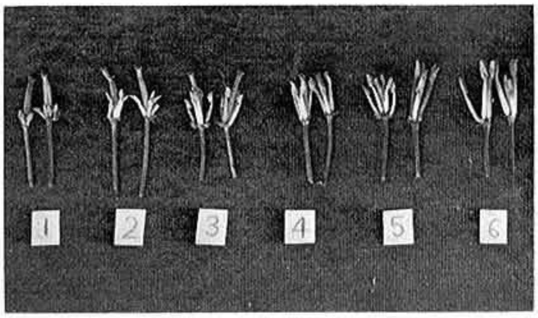


Plate 1. Phenotypic criterion of male sterility in rapeseed plants. The numeral shows relative position of anther to stigma. 1 and 2 are male sterile (ms); Stamens are short with undeveloped anthers and pollen grains are not produced or rarely produced 3 and 4 are partially male sterile (pms); Stamens are shorter than normal with abnormal anthers, and small quantities of pollen grains are produced in some or all anthers. 5 and 6 are male fertile (mf).

also included. Rapeseed cultivars could be classified into two groups according to the kind of the cytoplasm, namely "group S" which had male sterile-inducing cytoplasm, and the group N which had non-male sterile-inducing cytoplasm (normal).

Cultivars of group S without any restorer gene manifest male sterility, i.e. low relative position of anther to stigma, undeveloped anthers and very narrow petals. Cultivars of group S with any restorer gene(s) manifest partial male sterility or normal fertility. Cultivars in group N does not manifest male sterility independently of the presence of fertility restorer genes. These cultivars express normal relative position of anther to stigma, normal anther and wide petals, and therefore phenotype of all cultivars in this group show normal fertility.

Cultivars belonging to group S were subdivided into six classes, and cultivars of group N into three classes, according to the number and action of fertility restorer genes. They were named "S-O", "S-I", "S-II a", "S-II b", "S-III", "S-IV", "N-O", "N-I" and "N-II".

The male sterile line belongs to class S-O. Cultivars of classes S-I and S-II a posses a partial restoring ability to S cytoplasm. Cultivars of classes S-II b, S-III and S-IV posses a full restoring ability to S cytoplasm and these cultivars of these classes can be used as a fertility restoring cultivar. Hybrid between male sterile line and cultivars of class N-O shows complete male sterility and these cultivars can be used as sterility maintainers. Hybrid from cross of male sterile line and cultivar of class N-I or N-II shows partial male sterility. But they segregate male sterile plants as well, therefore cultivars of these classes can also be used as sterility maintainers.

Heterosis observed in rapeseed cultivars

The author compared the characters of 62 hybrids of intraspecific crosses in breeding trials to the characters of respective parents at the Rapeseed Breeding Center, Fukushima Agricultural Experiment Station during a period from 1954 to 1967. Performance of 48 hybrids was found superior to that of respective parents.

Also, performance of 131 hybrids between the male sterile line and 131 Japanese cultivars was compared to their pollen parents. Seed yields of 108 hybrids were found similar or superior to respective pollen parents.

Heterosis breeding of rapeseed plant in future

The major leading cultivars in Japan, namely Chosen-shu and European type cultivars, belong to the group S. These cultivars possess restorer gene(s). Although there are many cultivars of the group N, there exists only a limited number of male sterility maintaining cultivars without a fertility restorer gene (N-O). As a result, male sterility maintaining cultivars with single or two fertility restorer genes have to be used unwillingly for heterosis breeding for the present. New male sterile lines may be arised by successive back-crossing at least three times with maintaining cultivars having the least number of restorer genes (or hopefully without any). Then, they can be maintained by selfing with rarely formed pollen grains.

It is desirable for the new male sterile lines to have short plant height, be resistant to lodging and Sclerotinia rot disease and have good general combining ability. Until now, inheritance of fertility restoration has been studied only at Hiratsuka in Japan and Mokpo in Korea. In the future, these studies need to be carried out in other locations in Japan or in foreign countries having different climate.

References

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