Rape Breeding by Interspecific Crossing Between *Brassica napus* and *Brassica campestris* in Japan

By TOSHIO SHIGA

Chief, 2nd Laboratory of Genetics, Division of Genetics, Department of Physiology and Genetics, National Institute of Agricultural Sciences (Hiratsuka)

History of rape culture in Japan

Rape culture was widely disseminated in Japan about 400 years ago and *B. campestris* ($n=10$) was the species cultivated at that time. The indigenous place of this species and the history of its appearance in Japan are not known, but it is believed that the species came from China.

Rape species now cultivated extensively in Japan is *B. napus* ($n=19$) which was introduced in this country via two routes, one of which is believed to be the variety from Europe introduced into Japan about 100 years ago at the time Japan opened its doors to foreign countries, though there remains no authentic record about it. This variety came into cultivation under the name of "Chôsen-shu" in the western part of Japan.

The other is the so-called "Hamburg", a cold-resistant and very late-maturing variety from Germany which appeared in Hokkaido in 1886. This variety is cultivated in the cold regions of Hokkaido and Aomori in Japan.

The *B. napus* was also a late-maturing variety as compared with the local species, *B. campestris* at that time, but its acreage gradually increased in warm regions south of the Kanto district because of its greater resistance to the *Sclerotinia* rot disease, high oil content and high yield.

Rape breeding organization established by the Ministry of Agriculture and Forestry

The Ministry of Agriculture and Forestry started its rape breeding project in 1930 by establishing the Central Breeding Center at the Konosu Experiment Farm of the National Agricultural Experiment Station to carry out mating and breeding works in the F_1 and F_2 generations and the local breeding centers in charge of the test and selection in the F_3 and later generation at the Agricultural Experiment Stations at Fukushima, Fukui, Osaka and Fukuoka.

In 1947 the Central Breeding Center at Kōnosu and the local breeding centers at Fukui and Osaka were closed, and the works of these two local centers were transferred to the Motosu Agricultural Experiment Station. And, taking this opportunity, every breeding center began to handle all the works connected with breeding from mating to the registration of varieties.

Afterwards the works of the Motosu Experiment Station were shifted to the Tokai-Kinki Agricultural Experiment Station in 1951, and new breeding projects were started at the Hokkaido Agricultural Experiment Station in 1952 and at the Kanoya branch of the Kagoshima Agricultural Experiment Station in 1961.

But as the rape acreage rapidly decreased, the breeding work was given up in 1966 at both the Tokai-Kinki and Fukuoka Agricultural Experiment Stations and also at the Hokkaido Agricultural Experiment Station in 1968. Rape breeding is, therefore, being carried on at present at the two breeding stations of Fukushima and Kanoya and at a selection field of the Fujisaka branch, Aomori Agricultural Experiment Station.

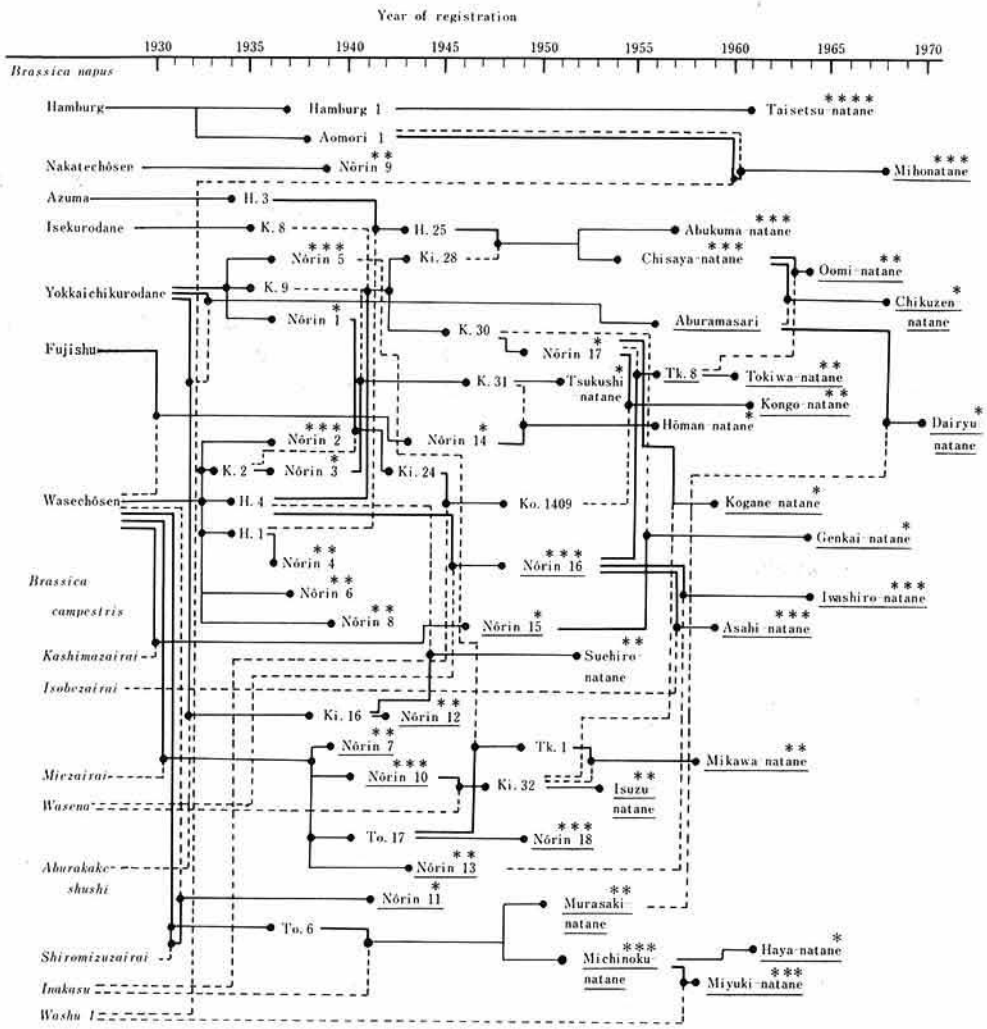

Fig. 1. Genealogical figures of registered rape (*Brassica napus*) varieties in Japan.

Note: 1. Varieties underlined were bred by interspecific cross or had both parents or a parent bred by interspecific cross.

2. Asterisks show breeding stations: *—Fukuoka Agri. Exp. Station, **—Osaka, Fukui, Motosu and Tokai-Kinki Agri. Exp. Stations, ***—Fukushima Agri. Exp. Station, ****—Hokkaido Agri. Exp. Station.
3. Names of parental lines are abbreviated: H.—Hokuriku, K.—Kyūshū, Ki.—Kinki, Ko.—Koshukei, Tk.—Tokai, To.—Tohoku.
4. Varieties connected with thick lines are female parents and those connected with broken lines are male.

Outline of rape breeding

When rape breeding commenced in Japan in 1930, the pure line selection and hybridization of *B. napus* derived from the "Chôsenshu" variety were begun simultaneously. The *B. napus* varieties used as parents for mating were newly introduced from various countries of Europe as well as some varieties originated in Chôsenshu and adapted to the climate of Japan. Those of *B. campestris* included new varieties brought in from China, Taiwan and India in addition to Japanese varieties which were extensively cultivated at that time. The combinations thus produced at Kōnosu during the 1930 to 1940 period were 286 intraspecific crosses of *B. napus* and 521 interspecific crosses between *B. napus* and *B. campestris*[11], while 410 intraspecific crosses and 144 interspecific crosses were obtained in total in various breeding stations in and after 1947.

The genealogical figures of the rape varieties bred at various breeding stations and registered at the Ministry of Agriculture and Forestry during 41 years from 1930 to 1970 are shown in Fig. 1.

The registered varieties are classified by years and breeding methods in Table 1 which shows that they total 41 in number and made up as follows: 10 varieties obtained by pure line selection, 16 varieties bred by intraspecific crossing and 15 varieties resulted from interspecific crossing.

Among the 16 varieties obtained by intraspecific crossing, nine have one or both parents which are varieties or lines bred by interspecific crossing[9]. These facts show that interspecific crossing played a very important part in rape breeding in Japan.

Breeding by interspecific crossing

The main object of rape breeding by interspecific crossing was the introduction of the early maturing character of *B. campestris* into the varieties of B. napus derived from "Chôsenshu" which had been brought to Japan because they were late-maturing varieties. In addition, such characters possessing greater resistance to high moisture of soil and less elongation of the pith in base stems were introduced into them.

Interspecific hybridization in *Brassicae* was studied by Kajanus[1),2),3)] and Sinskaya[10)] from the genetical and systematical viewpoints. Morinaga[5),6),7)] and U[12),13)] had independently investigated the genomes of *Brassicae*. Now, it is well known that B. campestris had A genome ($n=10$ and *B. napus* has AC genome ($n=19$). Artificially synthesized amphidiploid *napus* (AC) can be derived from crossing between *B. campestris* (A) and *B. oleraceae* (C), and duplication of those chromosomes of the F_1[13)].

Interspecific hybrid between *B. campestris* and *B. napus* had AAC genome (allotriploid, $n=29$). In meiosis, unpaired 9 chromosomes (C genome) may be separated unequally and produce unfunctional gametes[6),13)]. But, by chance, the F_1 plant produces normal gamete having napus-type genome (AC) and set F_2

Table 1. Number of registered rape varieties classified by years and breeding methods

Period	Pure line selection	*B. napus* × *B. napus*	*B. napus** × *B. napus*	*B. napus* × *B. campestris*	Total
1930 — 1939	8	0	0	1	9
1940 — 1949	0	2	0	7	9
1950 — 1959	0	5	2	6	13
1960 — 1970	2	0	7	1	10
Total	10	7	9	15	41

* Both parents or a parent of these varieties were bred by interespecific crosses between *B. napus* and *B. campestris*.

or backcrossed seeds. These seeds are expected to have some chromosomes of *A* genome from *B. campestris* variety which has favorable characteristics mentioned above. And a new type of *B. napus* could be created through this method.

Among the various varieties of *B. napus* used for interspecific crossing, those which produced a large number of registered varieties in the progeny were Wasechôsen, Yokkaichi-kurodane, etc., all of which were descended from "Chôsenshu".

Many of the registered varieties bred by the interspecific crossing, were also descendants of *B. campestris*, Japanese local varieties such as Miezairai, Wasena, Inakashu, Washu 1, etc. . . . Only two registered varieties were derived from a foreign variety of *B. campestris* (Aburakakeshushi).

The representative varieties bred by interspecific crossing are as follows; Murasaki-Miyuki-natane which were successfully improved in early maturity; Norin 13, Norin 16, Asahi-natane and Aburamasari which are new types never seen in *B. napus* so far, —Miho-natane[9] which is an early-maturing variety obtained by improving Aomori 1 which is a descendant of "Hamburg" brought to the cold regions of Japan.

Procedure of breeding by interspecific crossing

The breedings of Norin 16 and Asahi-natane, representative varieties obtained by interspecific crossing, were conducted on the scales as shown in Table 2.

The principal points of the breeding method were as follows:

1) Mating was accomplished by using *B. napus* with a larger number of chromosomes ($n=19$) as the female and *B. campestris* ($n=10$) as the male. Reciprocal crossing can be made but it is less fertile. Plants to be used for mating were potted before bolting. *B. campestris* generally matures very early, so, for mating, its flowering time must be regulated so as to be the same as that of *B. napus*.

Mating technique is not different from that for intraspecific crossing. But at least about 100 flowers must be pollinated for each combination because fertility is lower in this case than in intraspecific crossing. Such a number

Table 2. Scale of rape breeding by interspecific cross (examples)

(1) Norin 16 (Hokuriku 4×Wasena)

Number of families, lines and individuals planted and selected		Cross 1937	F_1 1938	F_2 1939	F_3 1940	F_4 1941	F_5 1942	F_6 1943	F_7 1944	F_8 1945	F_9 1946	F_{10} 1947	F_{11} 1948
Planted	Families					14	3	2	2	4	4	3	
	Lines				28	22	11	9	10	16	16	12	
	Individuals		30	2000	968	792	528	432	480	768	768	576	
Selected	Families					3	2	1	2	3	3	1	
	Lines				14	3	2	2	4	4	3	2	Regi-
	Individuals		30	28	22	11	9	10	16	16	12	8	stered

(2) Asahi-natane (Norin 16×Isobezairai)

Number of families, lines and individuals planted and selected		Cross 1950	F_1 1951	F_2 1952	F_3 1953	F_4 1954	F_5 1955	F_6 1956	F_7 1957	F_8 1958	F_9 1959
Planted	Families					4	2	2	2	2	
	Lines				11	12	8	8	8	8	
	Individuals		30	2000	396	432	432	240	240	240	
Selected	Families					2	1	2	1	1	
	Lines				4	2	2	2	2	2	Registered
	Individuals		30	11	12	8	8	8	8	8	

of pollinations will give 400–500 grains of hybrid seeds if the plants are normal in growth.

2) In the F_1 generation about 30 plants are grown (at a density of 3,000 individuals per 10 a). These F_2 plants are not sufficiently fertile when self-pollinated or pollinated in net so they are allowed to fructify under open condition. To obtain many plants of *B. napus* type in the F_2 generation, the crossing of F_1 individuals with *B. napus* should be promoted by growing plants of the same variety as the female parent around them or by backcrossing them with *B. napus* variety.

3) To raise F_2 plants, seeds with a diameter of 1.75 mm or more are selected for seeding because such seeds give a high percentage of *B. napus* type plants in the F_2 generation. The seeds are sown 15 × 15 cm apart in groups of several grains in the nursery.

The seedlings which have light green waxless glossy cotyledons and leaves and show vigorous growth at the beginning are thinned in nurseries together with the individuals which are deep green in color and slow in growth, leaving a single selected plant in each place.

In the F_2 generation about 2,000 individuals with serial numbers are planted in the field (at a density of 5,000 plants per 10 a) and examined for their bolting and flowering dates. They are self-pollinated in paraffin bags and examined for height and the number of primary branches in their maturity.

From among these plants about 100 individuals which are stronger in resistance to diseases, higher in fertility and have the plant and pod types characteristics of *B. napus* are selected to harvest at the time of maturity. They are examined for the total weight, seed weight, weight of 1,000 seeds, seed color and quality of seeds after drying.

Finally 10 to 30 individuals of *B. napus* type which have excellent characters suitable for breeding purpose are selected on the basis of the results of all examinations mentioned above in addition to their self-fertilities. The seeds obtained from these plants by self-pollination are used for raising F_3 individuals.

4) In the F_3 generation 36 individuals are grown for each line thus selected. But it is desirable to grow more plants if possible. The F_3 plants are numbered individually as the F_3 plants were, and 10 to 20 excellent individuals of *B. napus* type are selected from among them in the same way as in the G_2 generation. Through the selections in the F_2 and F_3 generations, the type of plants comes to resemble *B. napus*.

5) In the F_4 generation and later pedigree selection is made in the same way as in the case of breeding by intraspecific crossing. Special attention must be paid to the self-fertility of plants. An individual which shows high self-fertility in the F_5 generation may be *B. napus*.

If selection is intensively made to fix the type of *B. napus*, there is the possibility that good characters are lost in the course of selection. In parallel with the pedigree selection the selected lines are put to the preliminary performance and performance tests. They are also tested for many physiological characters and adaptability to the cultivating condition at each Agricultural Experiment Station.

The rape line superior in growth, productivity and other various characters are picked out by these tests and registered as varieties. Rape breeding by interspecific crossing generally takes a little more years from the first crossing to the registration of varieties than that by intraspecific crossing

Table 3. Number of rape varieties classified by generation numbers from intra or interspecific cross to registration

Breeding method	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	E_{14}	F_{15}	F_{19}	F_{25}	Total
B. napus × *B. napus*	1	2	4	1	1	1	4	—	2	—	—	16
B. napus × *B. Campestris*	—	—	3	3	2	—	1	2	2	1	1	15

as shown in Table 3.

Conclusion

The above-mentioned facts give an outline of rape breeding in Japan and an examination of the important part played by interspecific crossing in breeding in addition to an explanation of the breeding procedure. This breeding method seems to produce a good result when it is adapted by a country where *B. campestris* is widely cultivated and a rapid increase of its production by the introduction be *B. napus*.

References

1) Kajanus, B.: Genetische Studien an *Brassica. Zts. Ind. Abst. u. Vererbgsl.*, **6**, 137–139 (1912).
2) Kajanus, B.: Mendelische Studien an Rüben. *Fuhling Landw. Ztg.*, **61**, 142–149 (1912).
3) Kajanus, B.: Über Bastardierung zwischen *Brassica Napus* L. und *Brassica rapa* L.. *Zts. Pfl. Zücht.*, **5**, 265–322 (1917).
4) Mizushima, U.: Karyological Studies in *Brassiceae*. 1–112, Gihodo (1952). [In Japanese.]
5) Morinaga, T.: Preliminary note on interspecific hybridization in *Brassica*. *Proc. Imp. Acad. Jap.*, **4**, 620–622 (1928).
6) Morinaga, T.: Interspecific hybridization in *Brassica* I. The cytology of F_1 hybrids of *B. Napella* and various species with 10 chromosomes. *Cytologia*, **4**, 16–27 (1929).
7) Morinaga, T.: Interspecific hybridization in *Brassica* VI. The Cytology of F_1 hybrids of *B. juncea* and *B. nigra*. *Cytologia*, **6**, 62–67 (1934).
8) Shibata, M., *et al.*: On the new variety of the rapeseed plant "Iwashiro-natane". *Bull. Fukushima Pref. Agri. Exp. Station*, **1**, 65–73 (1965).
9) Shiga, T., *et al.*: A new variety of winter rape "Miho-natane"—Its breeding process and characteristics. *Bull. Fukushima Pref. Agri. Exp. Station*, **7**, 51–60 (1970).
10) Sinskaia, E. N.: Geno-systematical investigation of cultivated *Brassica*. *Bull. Appl. Bot. Pt. Breed.*, **17**(1), 1–166 (1917). [In Russian with English summary.]
11) Togari, Y. and Kanno, C.: Survey of cross matings in breeding of rape. *Jour. Kanto-Tosan Agri. Exp. Station*, **6**, 113–131 (1954).
12) U, N. and Nagamatsu, T.: On the differences between *Brassica campestris* L. and *B. napus* L. in regard to fertility and natural crossing. *Jour. Imp. Agri. Exp. Station*, **2**, 113–128 (1932).
13) U, N.: Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Jap. J. Bot.*, **7**, 389-452 (1935).